GENERATION OF AN IMMORTAL DIFFERENTIATED LUNG TYPE-II EPITHELIAL CELL LINE FROM THE ADULT H-2K<sup>tsA58</sup> TRANSGENIC MOUSE

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SUMMARY

This paper describes a new fully differentiated Type-II alveolar epithelial cell line designated T<sub>7</sub>, derived from transgenic H-2K<sup>tsA58</sup> mice, capable of being passaged as an immortalized cloned cell line in culture. H-2K<sup>tsA58</sup> mice harbor a temperature-sensitive (ts) mutant of the simian virus 40 (SV40) large tumor antigen (T antigen) under the control of the γ-interferon (INF)-inducible mouse major histocompatibility complex H-2K<sup>b</sup> promoter. When cultured under permissive conditions (33°C and in the presence of γ-INF) cells isolated from H-2K<sup>tsA58</sup> mice express the large T antigen, which drives the cells to proliferate. However, upon withdrawal of the γ-INF and transfer of the cells to a higher temperature (39°C), T antigen expression is turned off, the cells stop proliferating and differentiate. The T<sub>7</sub> cell line is a clonal cell line originally derived from a Type-II cell-rich fraction isolated from lungs of H-2K<sup>tsA58</sup> mice. The T<sub>7</sub> cells form confluent monolayers, and have a polarized epithelial cell morphology with tight junctions and apical microvilli. In addition, the T<sub>7</sub> cells have distinct cytoplasmic lamellar bodies, which become more numerous and pronounced when the cells are grown under nonpermissive conditions. The T<sub>7</sub> cells synthesize and secrete phosphatidylcholine and the three surfactant proteins, SP-A, SP-B, and SP-C. The T<sub>7</sub> cell line is unique in that it is the first non-tumor-derived Type-II cell line capable of synthesizing and secreting the major components of surfactant. Based on the criteria studied, the T<sub>7</sub> cell line is phenotypically very similar to normal Type-II cells. The T<sub>7</sub> cell line, therefore, should prove a valuable experimental system to advance the study of the cell biology/physiology of surfactant metabolism and secretion as well as serve as a model for other studies of Type-II cell physiology.

Key words: pulmonary; surfactant; lipid; protein; secretion; synthesis.

INTRODUCTION

Lung Type-II epithelial cells are present within the alveolar lining (Coalson, 1994) and produce the surface tension lowering lipoprotein complex, surfactant (Clements, 1957). Surfactant is a complex mixture of phospholipids and three proteins called surfactant protein A (SP-A), B (SP-B), and C (SP-C) (Weaver and Whitsett, 1991). The surfactant complex reduces surface tension at the air-liquid interface in the alveolus, which prevents alveolar collapse during expiration. When the surfactant is absent or is deficient, as in premature infants or in certain pathological states in adults, respiratory distress ensues (Avery and Mead, 1959; Hartog et al., 1991). The study of the cell biology and biochemistry of surfactant synthesis and secretion has been severely hampered due to the lack of a suitable in vitro cell model. One can isolate and culture primary Type-II cell lines have been derived from animals and human tissue (Smith, 1977; O'Reilly et al., 1988a; Mallampalli et al., 1992; Steele et al., 1992; Wikenheiser et al., 1993; Sen et al., 1994), but generally these have originated from tumors and most do not manifest sustained secretion of lamellar bodies, phospholipids, or the surfactant proteins. None of these cell lines are phenotypically close to Type-II cells. Moreover because of their transformed origin, it is uncertain whether these cell lines accurately reflect the cell biology/physiology of normal Type-II cells.

Polarized epithelial cells are notoriously hard to culture. However, using the transgenic H-2K<sup>tsA58</sup> (Immortal) mouse (Jat et al., 1991), several groups have recently generated differentiated immortal cell lines from epithelial tissues, such as colonic and small bowel epithelium (Whitehead et al., 1993), and bone (osteoclasts) (Chambers et al., 1993). These cell types had hitherto proved difficult, if not impossible, to culture. The transgenic H-2K<sup>tsA58</sup> (Immortal) mouse has inserted into its genome a construct consisting of the 5' flanking promoter sequence and the transcriptional initiation site of the mouse major histocompatibility complex H-2K<sup>b</sup> class I (MHC) gene coupled to the coding sequence of a temperature-sensitive form of the simian virus 40 (SV40) T antigen (Jat et al., 1991). The MHC gene promoter is not only widely active in a range of tissues but is also susceptible to additional induction by interferons. When cells from the Immortal mouse are isolated and grown under permissive conditions (33°C and with γ-interferon [γ-INF] present in the media) the SV40 T antigen is expressed causing the cells to...
proliferate. However, upon withdrawal of the \( \gamma \)-INF and transfer of the cells to a higher temperature (39°C), T antigen expression is turned off, the cells stop dividing, and, they proceed to differentiate.

In this paper we describe the generation and characterization of the Tc cell line, the first non-tumor-derived immortalized, fully differentiated Type-II cell line. This cell line was produced by dilution cloning of a Type-II cell-rich fraction produced from the lungs of transgenic H-2Kb-tsA58 (Immortal) mice. The Tc cell line is novel in that it expresses SP-A, SP-B, and SP-C. Furthermore, the Tc cells secrete all three of these proteins plus phosphatidylcholine, the major lipid component of the surfactant. In addition, the cells have a polarized epithelial cell morphology with distinct cytoplasmic lamellar bodies. The Tc cell line is phenotypically close to normal Type-II cells; therefore they will prove a valuable tool for the study of Type-II cell physiology.

**Materials and Methods**

Isolation and immortalization of Type-II epithelial cells from the lungs of transgenic H-2Kb-tsA58 mice. A modification of the technique described by Mason et al. (1997a) was used to isolate Type-II cells from the Immortal transgenic mouse lungs (Jat et al., 1991). In preliminary experiments on the parent nontransgenic strain CBA/Ca × C57BL, it was determined that a minimum of four mouse lungs were necessary to obtain a visible cell pellet. Consequently four Immortal mice were anesthetized and heparinized. The trachea was cannulated and the aorta transected to exsanguinate the mice while ventilation was maintained. The lungs were perfused through the pulmonary artery with a dextrose/salt solution until they appeared white and blood-free. The dextrose/salt solution was then used to lavage the lungs seven times, followed by lavage twice with a dextrose/salt solution plus 1.9 mM dextrose plus elastase. The airways and nonparenchymal tissues were dissected away and the rest of the lung placed in a deoxyribonuclease solution. Consequently four immortal mice were anesthetized and heparinized. The trachea was cannulated and the aorta transected to exsanguinate the mice while ventilation was maintained. The lungs were perfused through the pulmonary artery with a dextrose/salt solution until they appeared white and blood-free. The dextrose/salt solution was then used to lavage the lungs seven times, followed by lavage twice with a dextrose/salt solution plus 1.9 mM dextrose plus elastase. The airways and nonparenchymal tissues were dissected away and the rest of the lung placed in a deoxyribonuclease solution and minced into 1-mm cubes. Elastase activity was stopped with the addition of fetal bovine serum. The cell suspension was filtered through Teflon meshes of 100 and 20 \( \mu \)m, underlaid with fetal bovine serum and centrifuged at 130 \( \times \) g for 8 min. The lighter Type-II cells remain in the supernate. The supernate was again centrifuged to obtain a cell-rich pellet. To remove contaminating macrophages this pellet was resuspended in serum-free medium, placed on IgG-coated dishes and incubated for 1 h at 37°C. The supernate from the IgG-coated dishes was then centrifuged at 130 \( \times \) g for 5 min. The Type-II cell-rich pellets were resuspended in Dulbecco modified Eagle medium (DMEM) containing: fetal bovine serum, 2%; penicillin, 100 U/ml; streptomycin, 1%; amphotericin B, 250 \( \mu \)g/ml; \( \gamma \)-INF, 100 U/ml; transferrin, 5 \( \mu \)g/ml; epidermal growth factor, 0.025 \( \mu \)g/ml; insulin, 10 \( \mu \)g/ml; endothelial cell growth supplement, 7.5 \( \mu \)g/ml; endothelin-1, 40 nmol/ml; T3, 20 ng/ml; hydrocortisone, 0.36 \( \mu \)g/ml. These additives were selected because they are known to promote Type-II cell differentiation (Kresch et al., 1987; Massay et al., 1987; Van Scott et al., 1988; Mason et al., 1990; Sen et al., 1994). The cells were then plated on tissue culture plastic in low volume to promote cell adherence. Cells were incubated overnight in 10% \( \mathrm{CO}_2 \) at 33°C, the permissive temperature, which promotes cell proliferation. The cells grew rapidly to confluence on tissue culture plastic, and after trypsinization were split and replated. Cells were passaged eight times and then cloned cell lines were derived by limiting dilution cloning (Frosthley, 1991). Cells grown at the time of cloning were designated as passage 1.

For some experiments, cells were grown on collagen IV-coated plastic or on collagen-coated transwells (Becton Dickinson, Bedford, MA). For these experiments, the medium was supplemented with 2% Ultraspec G ( Biosprera, Cedex, France) (Yamaya et al., 1992). To study the effects of growth with an apical air surface on cell phenotype, cells were grown on collagen gels prepared as described by Dubois et al. (1997). Briefly, a stock solution of bovine dermal collagen (Vitrogen, Santa Clara, CA) was prepared by adding to eightfold of the collagen stock solution on 30-mm Millicell CM membranes (Millicare, Bedford, MA), the collagen was allowed to polymerize for 2 h at 37°C in a 5% \( \mathrm{CO}_2 \) incubator. The membranes were washed six times with MEM before plating with cells. After growth for ~48 h, the medium from the top of the collagen gels was removed to expose the apical cell surface to air. Cells were grown for 24–48 h under these conditions before harvesting for electron microscopy.

**Fig. 1.** RT-PCR was done on total RNA extracted by cesium chloride gradient from the Tc clone. Lane 1 is a negative control (without RNA) and represents no PCR product of the RT-PCR reaction without RNA. Lane 2 represents the PCR product resulting from the inclusion of RNA from Tc cells grown on tissue culture plastic at 33°C in the presence of \( \gamma \)-INF for ~5 d and then transferred to 39°C for 3 d without \( \gamma \)-INF. In lane 3, RNA from Tc cells grown to confluence on tissue culture plastic at 33°C in the presence of \( \gamma \)-INF was used. In lanes 4 and 5, RNA from mouse kidney or lung representing negative and positive tissue controls, respectively, was included in the PCR mixture. The amount of RNA used in each lane was standardized by optical density taken at 260 nm. In panel A, the expected 346 bp SP-A cDNA fragment was amplified from RNA from the Tc clone and from mouse lung. No SP-A cDNA amplification is seen in the negative lanes 1 or 4. In panel B the expected SP-B cDNA 260-bp fragment is seen in the Tc, lanes 2 and 3 and in lane 5, the positive control, but not in the negative control lanes 1 and 4. In panel C the expected SP-C cDNA 251-bp fragment is seen in the Tc, lanes 2 and 3 and in the positive control lane 5, but not in the negative control lanes 1 and 4. In all three panels an expected higher molecular weight DNA band was observed in lanes 2–4 reflecting contamination of RNA with genomic DNA and in lane 4 serves to show successful amplification in this negative control. Using primers for mouse \( \beta \)-actin cDNA, an expected 500-bp fragment was detected in cells corresponding to lanes 2–5 (data not shown).
without γ-INF for 3 d). Five-day old cultures were then pulsed with 10 μmol/l BrdU for 1 h before staining with anti-BrdU antibody crosslinked with alkaline phosphatase. The cells with BrdU incorporation as evidenced by nuclear staining were counted for both growth conditions.

Transepithelial resistance and impermeability of the T7 cell monolayer. Electrical measurements to determine trans epithelial resistance were performed on cells plated on collagen-coated transwells that had achieved confluence. The Millicell ERS voltmeter (Millipore) was used, and first, the resistance was measured in a ‘blank’, i.e., across a transwell membrane with no cells but with the same volume of medium in the top and bottom wells. The resistance was then measured across a confluent monolayer of cells grown on a transwell membrane. The difference between the readings in the blank and the cell sample was taken as the trans epithelial resistance. In addition, the impermeability of the confluent monolayer was demonstrated using tritiated inulin. After the addition of inulin (650 μCi/ml) to the medium in the top well, the transwells were incubated for 1 h before aliquots of medium present in the apical and basal wells were taken. The amount of inulin present in each aliquot of medium was then determined by liquid scintillation counting.

RNA extraction and reverse transcriptase-polymerase chain reaction. Each clonally isolated, conditionally immortalized, cell line was grown to conflu ence in two 100-mm dishes and total cellular RNA was isolated according to the method of Chomczynski and Sacchi (1987) and by subsequent cesium chloride gradient centrifugation. RNAs similarly prepared from mouse lung and mouse kidney (Ambion Inc., Austin, TX) were used as positive and negative controls whole mouse lung homogenates were used. For each sample, septum proteins including SP-C, as determined by RT-PCR, was chosen for further characterization.

Synthesis of SP-A, SP-B, and SP-C by T7 Type-II cell clone demonstrated by immunoblotting. T7 cell lines were grown on collagen IV-coated transwell filters under permissive conditions to near confluence, when some were transferred to nonpermissive conditions for 48–72 h. For some experiments, cells were treated with different doses of γ-INF—25, 50, or 100 μg/ml—when placed in permissive conditions, and after growth for 48–72 h or until near confluence, transferred to nonpermissive conditions. Cells were then harvested for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For positive controls whole mouse lung homogenates were used. For each sample the protein concentration was determined by spectrophotometric comparison with known standards (Lowry et al., 1951). Thus, an equal amount of protein from each sample was loaded and electrophoresed on one-dimensional 10 or 15% SDS-PAGE gels. Gels were electrophoretically transferred to 0.2-μm nitrocellulose (Hybond ECL, Amersham, Buckinghamshire, England), at 150 mA/cm² for 1.5 h, using a mini transblot electrophoretic transfer cell (BioRad, Hercules, CA). After overnight blocking at 4°C, filters were incubated at room temperature for 2 h in primary antisera at titers determined as optimal by preliminary experiments for each antisera used. The antisera for SP-A was kindly provided by Dr. David Phelps (Phelps et al., 1984). This affinity-purified antibody raised in rabbits against human alveolar proteinosis extract recognizes the 35 kDa SP-A and crossreacts with SP-A derived from the rat. The antibody to SP-B was a generous gift from Dr. Yasuhiro Suzuki (Suzuki et al., 1990) and was prepared in a hybrid cell (BBSE) prepared by
fusing a myeloma cell X63.Ag8.653 with spleen cells of the Balb/c mouse immunized with a surface-active fraction of porcine lung lavage. The antibody recognizes the 15 kDa SP-B and cross-reacts with the murine-derived antigen.

A second antibody to purified SP-B was obtained from the laboratory of Dr. Jeffrey Whitsett (Lin et al., 1996). This antiserum raised in rabbits recognizes the mature 8 kDa SP-B peptide. The antiserum to SP-C was kindly provided by Dr. Michael F. Beers (Beers et al., 1992, 1994). It was raised in rabbits against purified synthetic NproSP-C peptide and recognizes the 20-21 kDa, 16 and 6 kDa translational products of SP-C.

The incubation with the primary antiserum was followed by incubation with secondary goat anti-rabbit or anti-mouse antibody (depending on the primary antibody), conjugated to horseradish peroxidase (Amersham Corp., Arlington Heights, IL) at titers ranging from 1:5000 to 1:24,000 for 1 h. Bands were visualized by enhanced chemiluminescence (ECL) using the ECL kit (Amersham). To test for nonspecific reactivity of the secondary antibody with mouse antigens, some immunoblots were incubated with each secondary antibody, alone, for the same duration and at the same titer as used for the immunoblotting after the primary incubation. These immunoblots were then reacted with the ECL kit reagents to visualize any bands that resulted from nonspecific staining; no such nonspecific bands were seen.

Secretion of surfactant proteins SP-A, SP-B, and SP-C by T7 cell clone. Cells were grown on collagen IV-coated 6-well tissue culture plates, as described above, for 48-72 h. After transfer to nonpermissive conditions, the medium was collected and centrifuged at 200 x g for 10 min to pellet any floating cells. The supernatant was then transferred to a fresh tube and concentrated to one-third the volume in a speed vacuum concentrator. The concentrate was then boiled with SDS-PAGE loading buffer and separated on a 10% SDS-PAGE gel as described above. The separated proteins were then electroblotted onto a nitrocellulose membrane. The blot was then cut into strips; each strip was then probed with an antibody raised against SP-A, SP-B, or SP-C.

Electron microscopy. For electron microscopy, cells were grown to confluence in a 100 mm Petri-dish, on collagen-coated plastic, in transwells (Becton Dickinson) or on collagen gels as described above. The cells were grown for 48 h at 33°C in medium supplemented with γ-INF, and then for 48 h at 39°C in medium without γ-INF. The growth medium was aspirated and the cells rinsed with phosphate-buffered saline. This was discarded and the cells were rinsed with uranyl acetate and lead citrate and examined in a JEOL 100S electron microscope.

RESULTS

Identification of a Type-II cell line with expression of surfactant proteins SP-A, SP-B, and SP-C. A Type-II cell-rich fraction was isolated from lungs of adult transgenic H-2K-tsA58 mice and placed in primary culture. The cells were passaged eight times before clonal cell lines were derived by limiting dilution cloning. To determine which of the clonal cell lines possessed a Type-II cell-like phenotype, candidate clones were screened by RT-PCR for the expression of messenger RNA for SP-A, SP-B, and SP-C. Primer pairs were selected from the published cDNA and genomic sequences of SP-A (Kozhagen et al., 1992), SP-B (D’Amore-Bruno et al., 1991), and SP-C (Glasser et al., 1990). Each pair of primers spans a specific intron so that any genomic DNA contaminating the RNA preparation would produce a larger amplified DNA fragment than the expected cDNA fragment using the same primers. RT-PCR was also performed with primers from the mouse β-actin gene (Tokunaga et al., 1986) in order to verify the presence and integrity of the RNA samples. A number of the candidate Type-II clones were shown to express each of the three surfactant mRNAs (data not shown). One clone, designated T7, expressed the highest levels of mRNA for all three surfactant proteins and was chosen for further characterization. The results of the RT-PCR assays on this clone are shown in Fig. 1.

Synthesis of surfactant proteins SP-A, SP-B, and SP-C by T7 cell clone. Having established that the T7 clone expressed mRNA for all three surfactant proteins, we next determined whether these mRNAs were translated. To accomplish this, we screened, by Western blot analysis, T7 cell lysates for SP-A, SP-B, and SP-C proteins (Fig. 2). For these studies, mouse lung homogenates and a lamellar body preparation were used as positive controls. In addition, the antibodies used were fully characterized (see Methods section) and are monospecific for their designated antigen. The T7 cell lysates yielded a single 26–30 kDa band with the SP-A antiserum (Phipps et al., 1984). This band represents the mature fully processed form of SP-A. A band of similar size was observed as the mature SP-A protein in both the mouse lung and lamellar body preparations (data not shown). Both lung and lamellar body preparations revealed several larger molecular weight forms of SP-A as previously published (Phipps et al., 1984). In comparison, with antiserum to SP-B (Suzuki et al., 1986), the T7 cell lysate yielded a doublet at 42–44 kDa and a diffuse single band at 8–10 kDa. The doublet is the unprocessed pro-form of SP-B, while the 8–10 kDa band represents the mature fully processed SP-B peptide. In the SP-B blots a minor band was also observed at 28–29 kDa, this possibly is the carboxy-terminus of the pro-protein liberated by the first proteolytic cleavage of the unprocessed SP-B protein. All four of these SP-B protein bands were observed with this primary antibody in the mouse lung preparation (data not shown). Finally, with the SP-C antiserum, the T7 cell lysates yielded a doublet at 46–50 kDa and a very prominent band at 6–8 kDa. Both the mouse lung homogenate and lamellar body positive controls yielded a similar pattern of SP-C proteins (data not shown) as previously described (Beers et al., 1994). The 46–50 kDa band represents a multimere resistant to SDS dissociation that develops in vitro after boiling because of the extreme hydrophobicity of SP-C. A similar band was also detected by Beers et al. (1994) and immunoreactivity for this product was eliminated by competition with purified SP-C (Beers et al., 1994). The 6–8 kDa band represents a partially processed form of SP-C. Increased pro-
FIG. 3. Transmission electron micrographs of T7 cells grown on tissue culture plastic. (a) Cell growth under permissive conditions at 33°C for 72 h; reveals morphologic polarization characterized by apical filopodia and a smooth basal surface. The cells form intercellular junctions (short arrow) and contain cytoplasmic lamellar bodies (thin arrows), and basal lipid droplets (thick arrows, original magnification...
tein expression was seen in cells grown under nonpermissive conditions when a dose of 50 μg/ml of γ-INF was used to stimulate proliferation, and remained unchanged in cells grown under permissive or nonpermissive conditions and over several cell passages (5th through 85th, the latter being the last passage studied).

BrdU incorporation. T7 cells at both permissive and nonpermissive growth conditions were visualized by alkaline phosphatase-linked antibody to BrdU following BrdU incubation, in order to determine their level of DNA synthesis as a corollary to cell division. Cell proliferation was reduced by nearly 50% as evidenced by reduced BrdU incorporation in cells grown under nonpermissive conditions (mean 56 ± 8%; standard deviation, N = 6), at 33°C; mean 35 ± 6% (SD, N = 5) at 39°C.

Transepithelial resistance and impermeability of the T7 cell monolayer. The integrity of the T7 cell monolayer was assessed both by transmembrane electrical resistance and by impermeability to the inert radioactive macromolecule inulin.

The transepithelial resistance measurements ranged from 258 Ω cm² at 33°C to 145 Ω cm² at 39°C, depending on the degree of cell confluence. There was no leakage of radioactive inulin from the top to the bottom well even after 1 h incubation of medium in the top well with radioactive inulin. Both these findings indicate that the T7 cells form a confluent monolayer with intact tight junctions.

T7 cell morphology. Next, we examined the morphology of the T7 cells by light microscopy. The T7 cells maintained a polygonal epithelial cell morphology. The nuclei were round to oval, and the cytoplasm was granular. The granularity became noticeably more pronounced when the cells were grown under nonpermissive conditions (data not shown). When examined by electron microscopy the cells appeared to be polarized with apical microvilli and a smooth basolateral surface (Fig. 3). The T7 cells formed tight junctions and contained cytoplasmic multivesicular and lamellar bodies.

The number of lamellar bodies increased approximately fourfold when the cells were switched from permissive to nonpermissive culture conditions (mean at 33°C = 0.82 ± 1.28 and SD, N = 29 lamellar bodies/cell; mean at 39°C = 3.6 ± 2.5 and SD, N = 29 lamellar bodies/cell). In addition, lamellar bodies were significantly more numerous in cells that were exposed to fresh medium 4-6 h after fresh medium was added to culture dishes; reveals an increased number of cytoplasmic lamellar bodies (original magnification ×35,000).

DISCUSSION

The study of the detailed cell biology/physiology of the lung Type-II epithelial cell has been severely hampered by the lack of a reliable in vitro cell model. Freshly isolated Type-II cells can be placed in primary culture, but within 24-48 h these cells begin to dedifferentiate and lose their ability to manufacture and secrete surfactant (Shannon et al., 1992; Mason et al., 1997a). New primary culture methodologies have been developed; however, even with these, Type-II cells can only be kept differentiated and viable for 2-4 wk. Thereafter, the cells reach senescence and apoptosis. Although the new generation of primary culture methodologies offers many advantages over the older systems, primary cultures are not clonal and are not very hardy and often cannot withstand complex experimental manipulation. Many of these problems could be overcome if a suitable immortalized Type-II cell line existed.

Numerous attempts have been made to establish a differentiated respiratory Type-II cell line; however, these have only had a limited amount of success. In the rat, alveolar Type-II and pre-Type-II cell lines have been developed by transfer of the adenovirus E1A oncogene using a retroviral construct (Smith, 1977; Mallampalli et al., 1992). These cells do not manifest a sustained secretion of lamellar bodies, phosphatidylcholine, or the SPs A, B, or C. Several human Type-II cell lines have been developed from pulmonary adenocarcinomas (O’Reilly et al., 1988a). The NCI-H820 cell line reportedly contains lamellar bodies and synthesizes SP-A, SP-B, and SP-C,
but forms nonadherent cultures and so is not appropriate for studies examining secretory polarity. Furthermore, these cells are difficult to grow (Steele et al., 1992). The A549 cell line demonstrates some of the morphologic features of Type-II cells, but does not express surfactant proteins (Smith, 1977). The H441 cell line synthesizes only SP-A and SP-B, but not SP-C, so it is not clear that it really represents a Type-II cell line, as SP-C is considered a marker for Type-II cells (O'Reilly et al., 1988a, 1988b). Recently, distal respiratory epithelial cell lines from tumors generated in transgenic mice harboring the viral oncogene SV40 large tumor antigen under the transcriptional control of a promoter region from the human SP-C gene, have been developed (Wikenheiser et al., 1993). Of the several cell lines reported, the MLE-7 cell line reportedly manifests some morphologic features of Type-II cells and expression of SP-A, SP-B and SP-C. Since these lines were derived from lung tumors, aberrant development is a concern and these cell lines may not accurately reflect the cell biology of normal Type-II cells.

In the study reported here, we have employed the transgenic H-2Kb-tsA58 (Immortal) mouse (Jat et al., 1991) to generate a novel, fully differentiated Type-II cell line. The Immortal mouse harbors within its genome a transgene consisting of the 5' flanking promoter sequence and the transcriptional initiation site of the mouse SP-C gene, have been developed (Wikenheiser et al., 1993). Of the several cell lines reported, the MLE-7 cell line reportedly manifests some morphologic features of Type-II cells and expression of SP-A, SP-B and SP-C. Since these lines were derived from lung tumors, aberrant development is a concern and these cell lines may not accurately reflect the cell biology of normal Type-II cells.

In the study reported here, we have employed the transgenic H-2Kb-tsA58 (Immortal) mouse (Jat et al., 1991) to generate a novel, fully differentiated Type-II cell line. The Immortal mouse harbors within its genome a transgene consisting of the 5' flanking promoter sequence and the transcriptional initiation site of the mouse major histocompatibility complex H-2Kb class I (MHC) gene fused to the coding sequence of a temperature-sensitive mutant of the SV40 T antigen (SV40-tsAS58). The MHC gene is active in the majority of cells and is also susceptible to additional induction by interferons. Thus, when cells from the Immortal mouse are isolated and grown under permissive conditions (33°C and with γ-INF present in the media), the SV40 T antigen is expressed; this drives the cells to proliferate. However, upon withdrawal of the interferon and transfer of the cells to a higher temperature (39°C), T antigen expression is turned off and the cells stop proliferating and differentiate. Our studies have shown that differentiation is most pronounced when the dose of γ-INF used for stimulating proliferation at 33°C is 50 μg/ml.

Thus this model overcomes the difficulty of achieving stable integration of an exogenous oncogene into cellular DNA (Clement et al., 1990). Hence cells from the Immortal mouse can be driven to proliferate or differentiate in culture by turning the T antigen 'switch' on or off, with the addition or withdrawal of γ-INF, and by adjusting the temperature of the cultured cells. Because of these unique characteristics, Immortal mice have been successfully used to generate immortal cell lines of tissues that have hitherto proved difficult to culture such as colonic and small bowel epithelium (Whitehead et al., 1993) and bone (osteoclasts) (Chambers et al., 1993).

The T cell line described in this paper was derived from a Type-II cell-rich fraction produced from lungs of H-2Kb-tsA58 (Immortal) mice using a dilution-cloning strategy. Morphologically, the T cells closely resemble native Type-II cells. Specifically, the T cells are polarized with distinct cytoplasmic lamellar bodies. The number and size of the lamellar bodies did appear to increase when the T cells were grown under permissive conditions. In addition, there was an apparent increase in the number of lamellar bodies in the cells grown under nonpermissive conditions that were exposed to fresh media 4–6 h prior to harvesting. This observation suggests that the culture medium contains limiting quantities of the components required by the cells to produce lamellar bodies. The T cells were also shown to synthesize SP-A, SP-B, SP-C, and phosphatidylcholine, the four major components of pulmonary surfactant.
Furthermore, the cells were demonstrated to secrete constitutively the fully or partially processed forms of all three surfactant proteins and phosphatidylcholine, indicating that the T7 cells are able to manufacture and secrete surfactants. The T7 cells demonstrate differentiated secretion of surfactant proteins and phospholipids at both 33 and 39°C. While the number of lamellar bodies increase at the nonpermissive temperature, the amounts of surfactant proteins exhibited by Western blot appear comparable. The most significant observed difference in the T7 cell line at 39°C is the progressive loss of cell proliferation and eventual cell death, which do not occur at 33°C. Thus this line is the first, non-tumor-derived, immortalized and clonal Type-II cell line that appears to be able to differentiate and is capable of synthesizing and secreting surfactant. Unlike previously described tumor-derived Type-II cell lines, the T7 cell line does not appear to have lost any Type-II cell specific biochemical or morphological features, and unlike primary Type-II cell cultures, T7 cells can be maintained indefinitely in cell culture and clonally expanded with ease.

The unique Type-II cell line T7 synthesizes both surfactant lipids and the proteins SP-A, SP-B, and SP-C. The lipids and all three surfactant proteins play an important role in the assembly of the surface-active lipoprotein complex, the lamellar body, and/or its extrusion from the cell. Recent ultrastructural and immunohistochemical observations in the lungs of SP-B-deficient children and transgenic mice suggest that SP-B plays an important role in the assembly of the lamellar body, its intracellular trafficking, and in the metabolism of the surfactant proteins SP-A and SP-C (Nogee et al., 1993; deMello et al., 1994a, 1994b; Clark et al., 1995). Now, with the availability of a normal (non-tumor-derived) and clonal cell line that produces both surfactant lipids and proteins, these molecular mechanisms can be more clearly defined. The insight into Type-II cell biology that these studies will provide, could have a significant impact on the management of respiratory disorders that result from Type-II cell dysfunction, such as the adult and infantile respiratory distress syndromes.

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