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Cystic fibrosis, lung infections, and a human tracheal antimicrobial peptide (hTAP)

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Abstract In order to understand how lungs of healthy people, unlike those of cystic fibrosis (CF) patients, are protected against bacterial infections such as *Pseudomonas aeruginosa*, the following three key findings were made. First, *P. aeruginosa* do not multiply when planted onto tracheal epithelial cells from healthy humans but do so profusely on cells from ΔF508 CF patients. Second, some bacteria bind, and gain entrance into CF cells, even at a physiological salt concentration (104 mM). Third, human tracheal epithelial cells express an ~4 kDa peptide (hTAP), which is known in its bovine form to exhibit bactericidal action against *P. aeruginosa*. A model is proposed depicting both how normal epithelial cells, in a first-line self defense mechanism, may be protected against bacterial infection and how this mechanism may fail during the initial stages of CF.

Key words: Cystic fibrosis; CFTR; Lung infection; *Pseudomonas aeruginosa*; Antimicrobial peptide; Defensin

1. Introduction

The disease cystic fibrosis is an inherited disorder that affects approximately 1 in 2000 people in the United States and Canada. Individuals with severe cases frequently die before the age of 30. The disease is characterized by lung dysfunction, pancreatic insufficiency, and increased sweat chloride concentration. Cystic fibrosis is caused by mutations in the CFTR protein, a chloride channel [1]. The most common mutation, the deletion of Phe-508 (ΔF508), results in chronic infections by *Pseudomonas aeruginosa* in the lungs of CF patients.

Despite much recent work, both on the first nucleotide binding domain of the CFTR protein in which the ΔF508 mutation resides [2–6], and on the pathogenesis of CF [7–10], the mechanism by which CF patients acquire chronic lung infections is unclear. In one recent report [9] it is concluded that infectious *P. aeruginosa* enter normal tracheal epithelial cells but not CF cells, the implication being that a normal cell's ability to combat bacterial infection is dependent on bacterial entry, which in turn is dependent on normal CFTR function. In a second intriguing report [10], it is suggested that, rather than 'engulfing' invading bacteria, normal tracheal cells secrete an antibacterial agent, a defense mechanism rendered impotent in CF cells, presumably because of a higher NaCl concentration in the airway fluid resulting from mutated CFTR. Finally, in a third study, [11] evidence is provided that *P. aeruginosa* interact directly with normal and CF cells via asialoganglioside 1, the postulated receptor, and that increased amounts of this receptor in CF cells may help explain the pathogenesis. Although there are apparent differences among these studies, the general implication is that *P. aeruginosa* have a propensity to localize more near CF cells than normal cells.

Below we briefly summarize studies carried out to understand the cellular and biochemical basis of CF pathogenesis. These studies entail both an extensive ultrastructural analysis of several thousand normal and CF human epithelial cells exposed to *P. aeruginosa*, and the cloning and sequencing from human tracheal epithelial cells of a small peptide known to exhibit bactericidal action against these bacteria. Collectively, these studies provide a novel model to explain how normal tracheal epithelial cells in a 'first-line defense' process combat bacterial infections while CF cells fail to do so.

2. Materials and methods

2.1. Materials

The NHBE primary cells, which are predominantly tracheal epithelial cells, and BEGM (a modified version of LHC8 medium) were from clonetics. HTE and CFTE cell lines were gifts from Dr. D. Gruenert of USCSF. The CFPAC cell line, and the mucoid form of *P. aeruginosa*, obtained from the sputum of a CF patient, were from ATCC. The doubling time of *P. aeruginosa* in tryptic soy broth medium (Difco) was about 20 min. The RNA PCR Kit, and the template and primers used in studies of hTAP were from Perkin Elmer. The Puregene DNA Isolation Kit was obtained from Gentra, and both Dulbecco's phosphate-buffered saline and trizol were from Gibco BRL. The Oligotex Direct mRNA Purification Kit, Quiaquick Gel Extraction Kit, and Plasmid Miniprep Kit were purchased from Quiagen. PCR primers were synthesized in the Johns Hopkins University core facility. The microwave oven (Model 3400) and the 200 mesh copper grids were from Pelco.

2.2. DNA sequencing

DNA templates for the automated DNA sequencing reaction were obtained after purifying the PCR amplified products from 3% low melting point NuSieve GTG agarose gels. For NHBE, HTE, and CFPAC cells, total RNA was isolated from these cells and respective cDNAs were synthesized for RT-PCR according to the method described under Fig. 3. In the case of the CFTE cells, the genomic DNA was prepared from the cell lysate by use of Gentra's Purengene DNA Isolation Kit which was used as the template for PCR synthesis of the F508 region of CFTR. The sequence of the primers employed for the PCR were as follows: 5′ primer: base G1548–base T1565 (18-mer); 3′ primer: base G1685–C1708 (24-mer).

2.3. Infection of epithelial cells with P. aeruginosa and transmission electron microscopy

The trypsinized epithelial cells (~10^6 cells) were seeded onto poly-

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Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; HTE, human tracheal epithelial; CFTE, cystic fibrosis tracheal epithelial; CFPAC, cystic fibrosis pancreatic; NHBE, normal human bronchial epithelial; BEGM, bronchial epithelial growth medium; PBS, phosphate-buffered saline solution; TEM, transmission electron microscopy; PCR, polymerase chain reaction; hTAP, human tracheal antimicrobial peptide; APs, antimicrobial peptides.

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carbonate Snapwell membranes (growth area=1 cm^2) and cultivated for 5 days in a 37°C incubator supplemented with 5% CO_2. Then, freshly grown *P. aeruginosa* (A_{600nm}=0.5) were diluted with BEGM (modified version of LHC8 medium containing 104 mM NaCl), to provide bacteria to epithelial cell ratios of 1:300, 1:30, or 1:1 in 100 μl. This volume was more than sufficient to cover all cells, and an increase to 500 μl did not alter the outcome of experiments described below. The apical sides of epithelial cells was exposed to bacteria for 10 h at 37°C. After this infection period, cells on the membrane were rinsed with 1×PBS and fixed with 2 ml of paraformaldehyde plus 2% glutaraldehyde in 1×PBS, pH 7.4, with the top plate covered. The cells in the fixative above were placed in a water bath inside a microwave oven and samples were microwave pulsed according to [12,13] with a 10 s pulse/20 s rest/10 s pulse, maintaining 100% power at each step. The fixative temperature did not exceed 30°C. Cells were allowed to remain in the fixative for an additional 5 min and briefly rinsed in PBS, followed by a rinse with 0.2 M sodium cacodylate.

Samples were microwave-postfixed as before in $K_3Fe(CN)_6$ reduced in 1% osmium tetroxide, rinsed in distilled water, and stained in filtered 1% aqueous uranyl acetate for 15 min. Treated cells on the membrane were quickly dehydrated in a graded series of ethanol washes, infiltrated with eponate, and cured first in an oven for 3 days at 37°C and then overnight at 60°C. Ultrathin sections (80 nm) were cut horizontally or vertically on a low angle Diatome diamond knife and collected on 200 mesh copper grids. Sections were additionally stained with 2% aqueous uranyl acetate for 25 min, rinsed, and allowed to dry. Samples were viewed on a Zeiss 10 B Transmission Electron Microscope operating at 60 kV.

2.4. Expression and sequence of hTAP

Total RNA or mRNA from NHBE and T84 cells grown to confluency on T-75 flasks was prepared by employing Trizol and an Oligotex Direct mRNA Purification Kit, respectively. According to the protocol provided by Perkin Elmer's RNA PCR kit, cDNAs were synthesized from the RNA prepared as above and were used as templates for RT-PCR under the following conditions: reverse transcription reaction: 10 min at 25°C (ambient temperature), 15 min at 42°C, 5 min at 99°C, and 5 min at 4°C; PCR: 2 min at 95°C, one cycle; followed by 35 cycles of 1 min at 95°C, 1 min at 55°C, and 3 min at 72°C; finally, 10 min at 72°C, one cycle. The RT-PCR amplified products were then analyzed by agarose gel (1.4%) electrophoresis, after which the bands having the expected molecular mass of 220 bp were excised from the gel, purified using the method of Qiagen's Qiaquick Gel Extraction Kit, and finally ligated into TA cloning sites of the TA cloning Kit Vector pCR II. TA Cloning One Shot competent cells were used and transformed by the ligation mixture above. The resultant recombinant plasmid was purified by use of Qiagen's Plasmid Miniprep Kit and 3 μg was subjected to automated DNA sequencing analysis with the T7 promoter sequence as the sequencing primer.

3. Results and discussion

In order to understand better how normal human epithelial cells combat bacterial infections whereas CF cells do not, we studied a normal human tracheal epithelial cell line (HTE) and two cell lines from humans afflicted with CF, i.e. CFTE cells from the trachea and CFPAC cells from the pancreas. The ΔF508 genotype of CFTR and CFPAC cells was confirmed (Fig. 1D and E, respectively) by use of PCR and automated DNA sequencing [14–16]. The DNA sequence of CFTR in the HTE cell line and in NHBE primary cells, also used in this study, was normal in the F508 region (Fig. 1C and B, respectively).

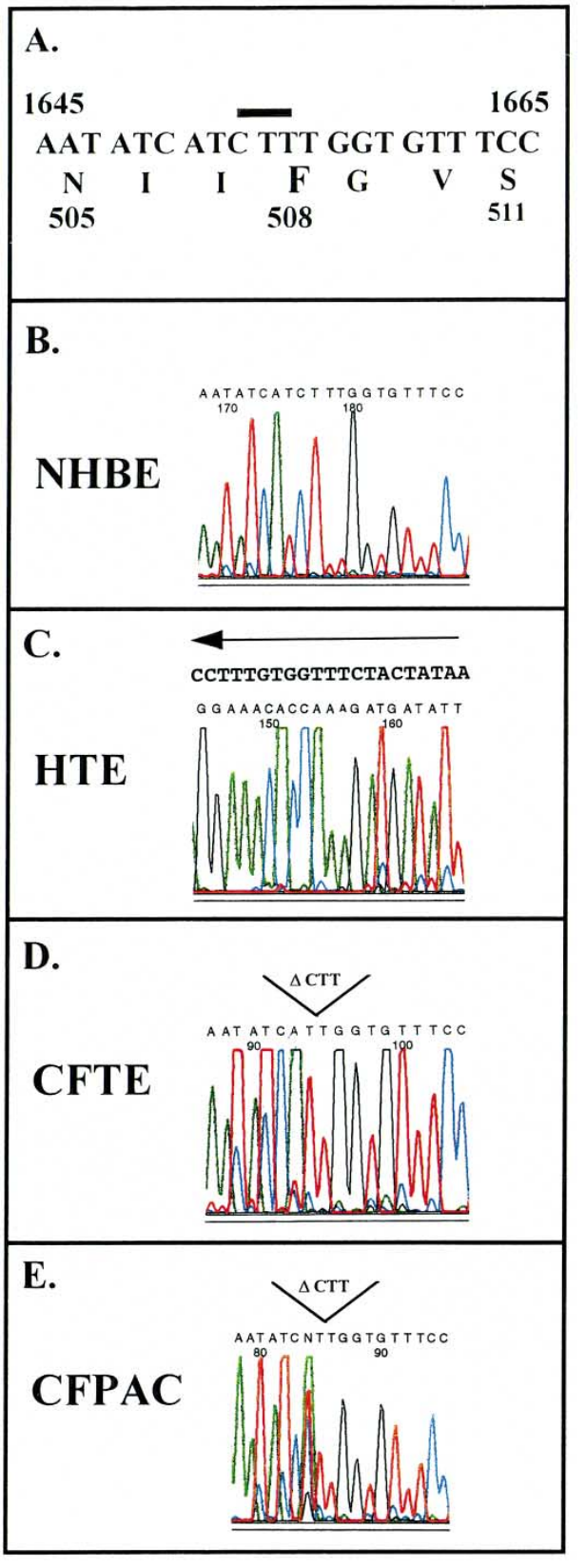


Fig. 1. Confirmation of the genotypes of the cells employed by DNA sequencing near the F508 region. (A) The DNA sequence of CFTR near the F508 region (from N505 to S511) is shown to depict the ΔF508 mutation resulting from deleting C TT (represented as a bar across the sequence). NHBE (B) and HTE (C) cells contain the predicted wild-type CFTR DNA sequence, near the F508 region, whereas CFTE (D) and CFPAC (E) cells show that C TT has been deleted without generating a frame shift, thus resulting in the ΔF508 mutation. The antisense DNA is sequenced in HTE cells and the arrow indicates the direction of the sense DNA.

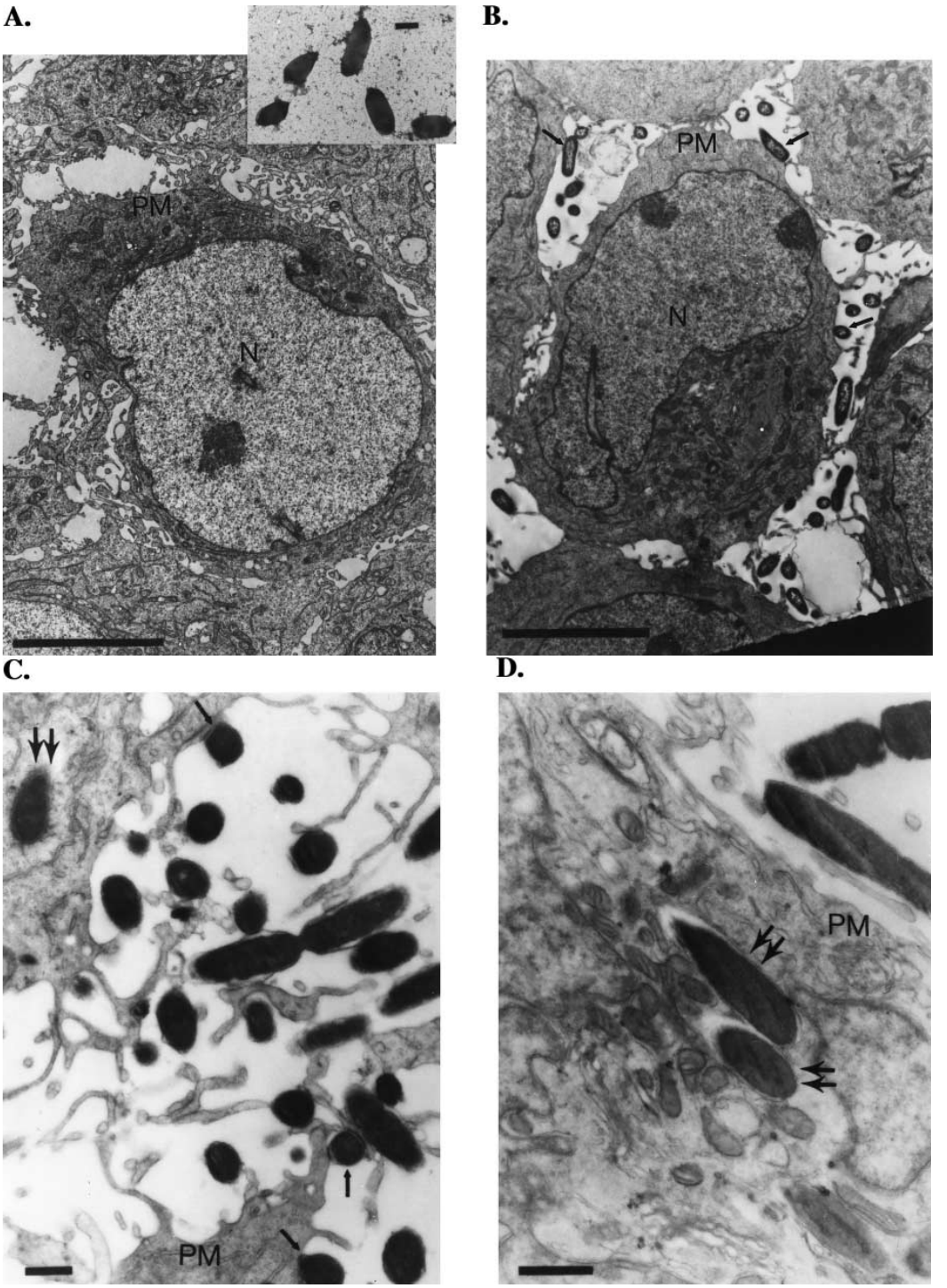


Fig. 2. Ultrastructural analysis by TEM on horizontally cut thin sections. (A) Normal (HTE) cells after 10 h exposure to *P. aeruginosa*. The bacterial infection has cleared and no free floating or bound bacteria are observed. N and PM designate the nucleus and plasma membrane, respectively. (Inset) *P. aeruginosa* observed after 10 h in the absence of HTE cells. The scale bar represents 10 μm in (A,B), and 1 μm in the inset. (B–D) CFTE cells (ΔF508 CF tracheal cells) infected with *P. aeruginosa*. Binding of the bacteria to CFTE cells (single arrows) and their growth on or near the microvilli of these cells is clearly observed (B,C). CFTE cells in which *P. aeruginosa* gained entrance are shown in C,D. Scale bars = 1 μm. (E,F) CFPAC cells (ΔF508 CF pancreatic cells) after infection with *P. aeruginosa*. CFPAC cells were unable to clear the bacterial infection. Rather, *P. aeruginosa* were found to bind and enter these cells. The scale bar is 10 μm in length (E). Higher magnification (F, scale bar = 1 μm) clearly shows that *P. aeruginosa* both bind (single arrows) and enter (double arrows) CFPAC cells. (See Table 1 for bacteria/cell infection ratios for A–F.)

Following the establishment of cell genotypes, bacterial infection experiments were conducted to determine how normal and CF cells interact with *P. aeruginosa*. Cells were grown on a permeable filter support (Snapwell polycarbonate membrane) to improve cell differentiation. Subsequently, they were exposed to *P. aeruginosa* from the apical side for 10 h at 37°C. Bacteria were diluted in BEGM, a modified version of LHC8 medium having a NaCl concentration of 104 mM,

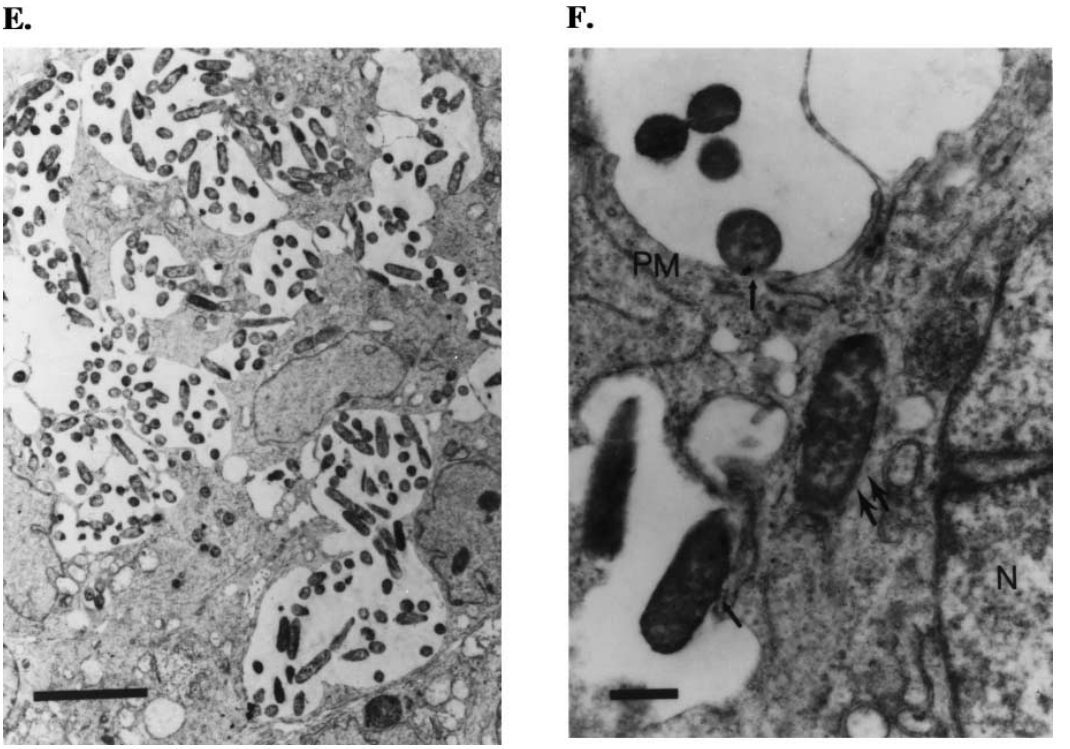


Fig. 2 (*continued*).

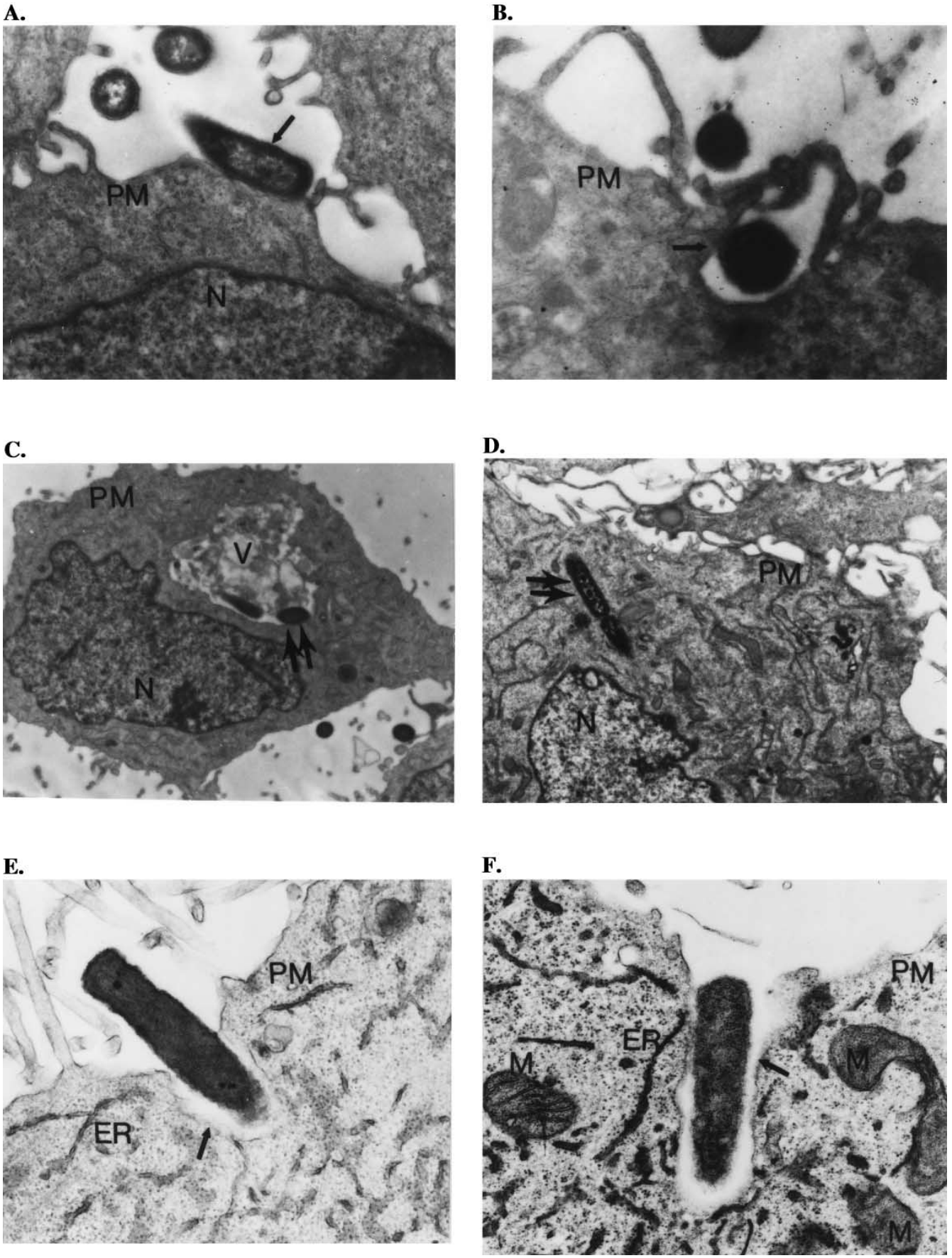
considered to be in the normal physiological range for airway surface fluid [17,18]. Inspection of horizontally cut thin sections of the normal cells by TEM after a 10 h exposure to *P. aeruginosa*, as shown in Fig. 2A, did not reveal any free floating bacteria, although in companion experiments conducted in the absence of epithelial cells numerous bacteria were present (Fig. 2A, inset). Furthermore, in micrographs not presented here, some normal cells were seen to continue with mitosis. These experiments demonstrate that normal tracheal epithelial cells exhibit the capacity to clear ('kill') these bacteria during a 10 h incubation period while maintaining growth. Even when the infection ratio of *P. aeruginosa* to normal cells was increased from 1:300 (Fig. 2A) to 1:1, these cells were able to resist the bacterial attack and completely clear the surrounding bacteria. In sharp contrast, the CF ΔF508 cells (CFTE) were unable to clear the surrounding bacteria. Consequently, these bacteria multiplied substantially. Some became bound to the epithelial cells, interacting with the microvilli, (Fig. 2B–D), and some even gained entrance to the cells during a 10 h infection period. Similarly, cells from the other CF ΔF508 line (CFPAC) were also unable to mount a defense against invading bacteria during a 10 h incubation period. Again, *P. aeruginosa* surrounded the cells with a significant number actually gaining entry into the cell (Fig. 2E,F).

In order to ensure the validity of our conclusions, the relative capacities of several thousand normal and CF cells to bind and permit bacterial entry as visualized by TEM of horizontally cut thin sections, was monitored and quantified as shown in Table 1. These extensive studies clearly demonstrate a dramatic difference between the capacity of normal and CF cells to resist *P. aeruginosa*. Thus, binding and entry of these bacteria into CF cells was significant (20% in CFTE cells and 50% in CFPAC cells), in sharp contrast to normal cells, where after 10 h, bacterial binding could not be detected in ∼4000 cells examined, although 2 cells showed bacterial entry. It is interesting to note that multiple bacteria tend to surround or target particular CF epithelial cells at any one time rather than making a concerted attack on the whole population of

Table 1
Quantification of epithelial cells having bound and entered *P. aeruginosa*

Cell type	Number of cells examined	Infection ratio: number of bacterial cells to number of epithelial cells	Number of cells with bound *P. aeruginosa*	Number of cells with entered *P. aeruginosa*
HTE	∼4000	1:1	0	2
CFTE	∼2000	1:1	∼300	∼100
CFPAC	∼2000	1:30	∼700	∼300

Cells were infected with *P. aeruginosa* exactly as described in Fig. 2. Horizontally cut thin sections of epithelial cells on 200 mesh copper grids were scored by viewing with a Zeiss 10B transmission electron microscope (TEM). Of the total number of epithelial cells examined (first column), the percentage observed to have bacteria bound (fourth column) plus that observed to have bacteria entered (fifth column), was 20% (CFTE cells) and 50% (CFPAC cells). An epithelial cell having either one or many bound bacteria was scored as one (fourth column). Also, an epithelial cell having either one or many entered bacteria was scored as one (fifth column). Significantly, multiple bacteria tend to surround or target particular CF epithelial cells at any one time rather than making a concerted attack on the whole population of cells. (Approximate signs are used because some small degree of error is expected when counting several thousand cells on multiple EM grids by eye).


A.
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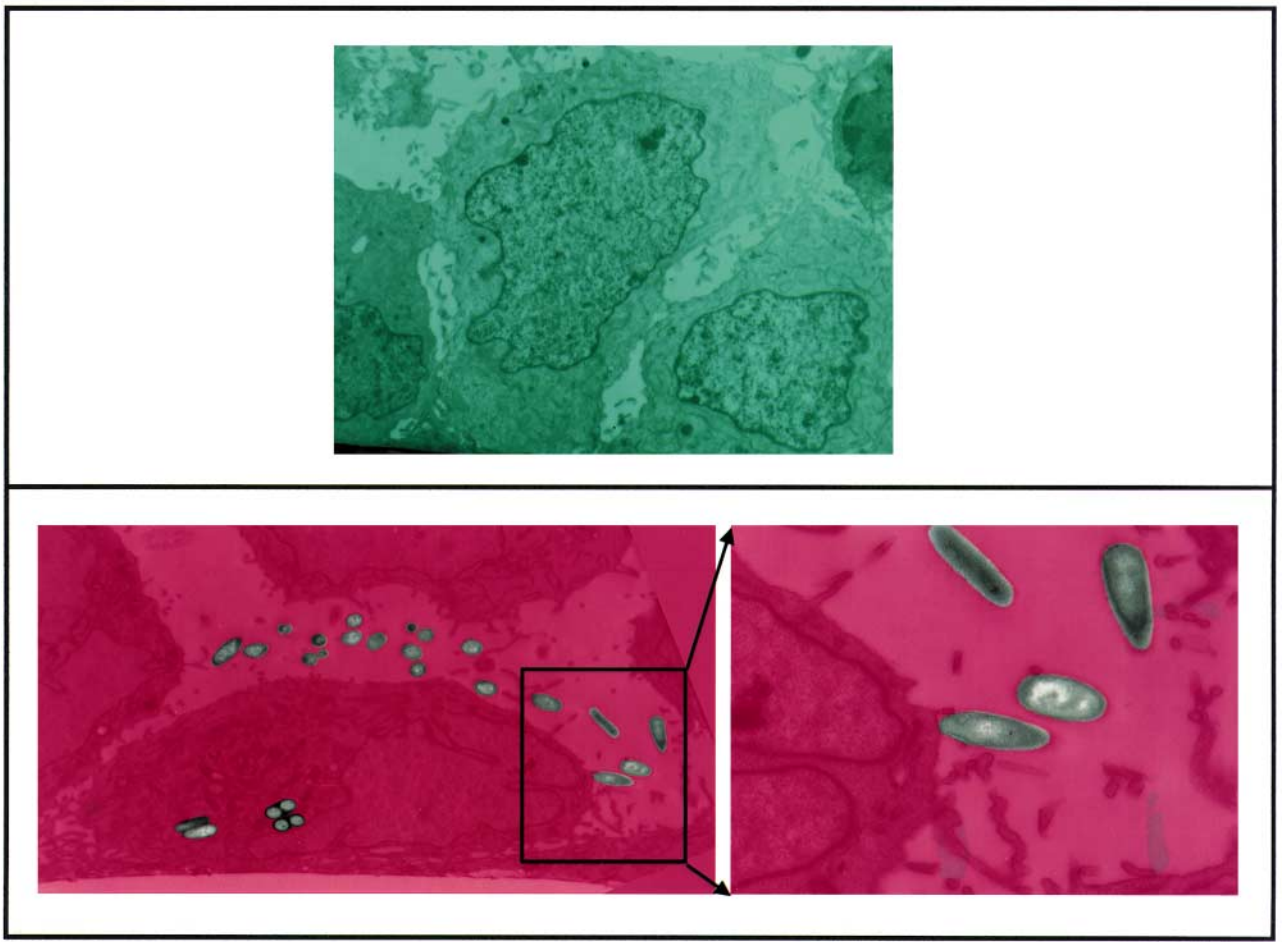

Fig. 4. Ultrastructural analyses by TEM on vertically cut thin sections. Normal cultured tracheal epithelial cells after 10 h exposure to *P. aeruginosa* completely clear the bacterial infection (panel in green), while cultured ΔF508 CF cells (CFTE cells, magenta) permit binding, entry, and multiplication of these bacteria. The magnification is, respectively, for normal cells and CF cells: 3539× and 2888×. The expanded bottom right panel shows binding of a *P. aeruginosa* bacterium (magnification: 10 193×) to a CFTE cell. See Section 2 for details. Artificial coloration was computer generated in order to enhance the contrast of the bacteria relative to the cellular background.

cells. At this time the reason for this is unknown but it may be that other bacteria are alerted to a CF cell that has been randomly penetrated by a single bacterium, or that bacteria can 'sense' the weakest members of the CF cell population, perhaps cells in the process of dying.

Fig. 3 displays additional electron micrographs from our TEM studies of horizontally cut thin sections of CF and normal cells after infection with *P. aeruginosa*. Several points seem pertinent. First, three types of interactions of these bacteria with CF cells are commonly observed: binding (Fig. 3A), encirclement by microvilli (Fig. 3B), and entrapment inside the cells (Fig. 3C,D). Secondly, intracellular entrapment occurs either within vacuoles (Fig. 3C) or within the cytoplasm, usually in contact with an intracellular organelle (Fig. 3D). The latter data leave no doubt that the bacteria have entered the cell. Finally, although deep invaginations formed by entering bacteria may have occurred in some cases prior to intracellular entrapment, this was not observed by careful examination of ∼4000 infected CF cells. In contrast, formation of deep invaginations by bacteria was clearly observed in a few normal cells (Fig. 3E,F) although the entry of bacteria (i.e. actual entrapment inside the cells) was a very rare event (only 2 in ∼4000 cells, Table 1). These findings would suggest that normal cells have a very high resistance to bacterial entry, whereas this resistance has been severely compromised in CF cells.

In order to ensure further the validity of our conclusions, we performed TEM on vertically cut thin sections of both normal and ΔF508 CF tracheal epithelial cells. In contrast to horizontally cut thin sections (Fig. 2A–F), vertically cut thin sections include the apical side of cells, thus allowing surface interaction between epithelial cells and bacteria to be

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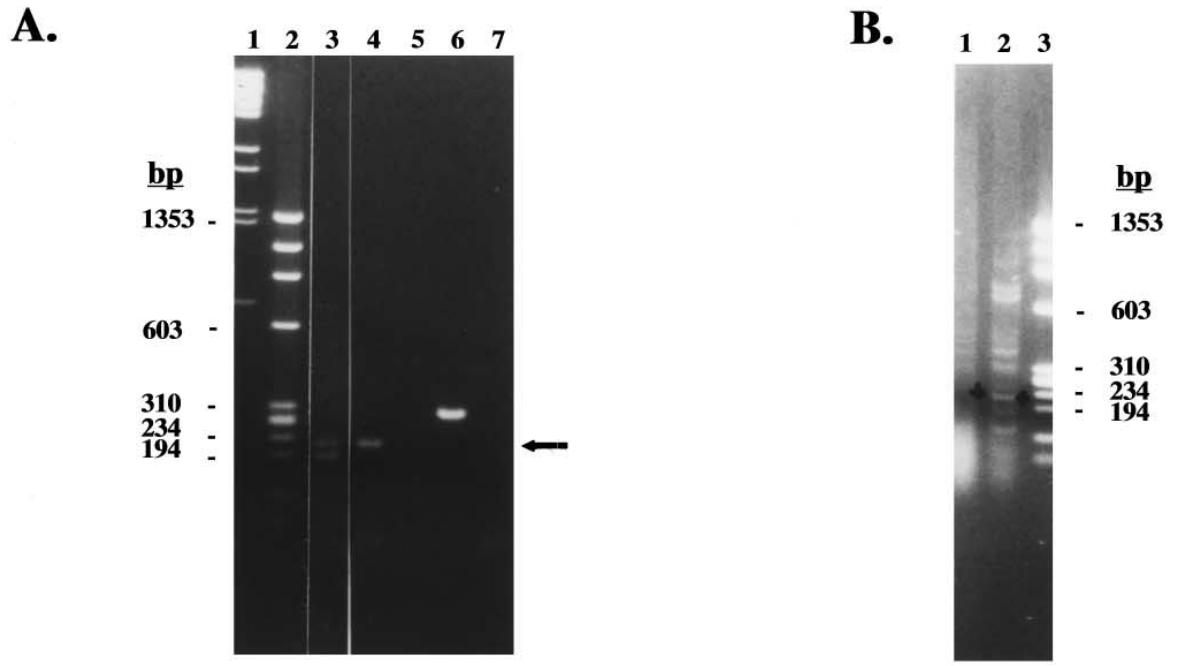
Fig. 3. Selected display of electron micrographs from normal and CF cells following 10 h exposure to *P. aeruginosa*. (A) A single *P. aeruginosa* bacterium bound to the plasma membrane of a CFTE cell (magnification = 14 800×); (B) a single bacterium bound to the plasma membrane (PM) of a CFTE cell and encircled by microvilli (magnification = 23 040×); (C) two bacteria entrapped inside a CFTE cell within a vacuole (V) (magnification = 6320×); (D) a single bacterium in contact with a mitochondrion inside a CFTE cell (magnification = 6122×); (E,F) bacteria penetrating HTE cells (normal cells) forming deep invaginations (magnifications = 26 730× and 25 740×, respectively). Where indicated, PM = plasma membrane, N = nucleus, V = vacuole, ER = endoplasmic reticulum, M = mitochondrion. Arrows designate *P. aeruginosa*. Two arrows indicate that the designated bacteria are within the cell.



C.

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hTAP                                                GCGCTCCTCTTCCTGGTCCTG
                        5′Primer                    :::::::::::::::::::::
bTAP CGCGCGAATTCCAGCATGAGGCTCCATCACCTGCTCCTCGCGCTCCTCTTCCTGGTCCTG  60
                       MetArgLeuHisHisLeuLeuLeuAlaLeuLeuPheLeuValLeu   15

hTAP TCTGCTTGGTCAGGATTTACTCAAGGAGTAGGAAATCCTGTAAGCTGTGTTAGGAATAAA
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
bTAP TCTGCTTGGTCAGGATTTACTCAAGGAGTAGGAAATCCTGTAAGCTGTGTTAGGAATAAA 120
     SerAlaTrpSerGlyPheThrGlnGlyValGlyAsnProValSerCysValArgAsnLys   35

hTAP GGCATCTGTGTGCCGATCAGGTGTCCTGGAAGCATGAAACAGATTGGCACCTGTGTTGGG
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
bTAP GGCATCTGTGTGCCGATCAGGTGTCCTGGAAGCATGAAACAGATTGGCACCTGTGTTGGG 180
     GlyIleCysValProValArgCysProGlySerMetLysGlnIleGlyThrCysValGly   55

hTAP CGG
     :::                 3′Primer
bTAP CGGGCAGTAAAATGCTGTAGAAAGAAGTAACTCGAGGCGC                     220
     ArgAlaValLysCysCysArgLysLys***                                64
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Fig. 5. Expression of hTAP in NHBE cells. (A) An agarose gel (1.4%) demonstrating the RT-PCR amplified product of hTAP as depicted by the arrow (220 bp from total RNA, lane 3) and from mRNA (lane 4). Control experiments were performed in the absence of templates (lanes 5,7) and in the presence of the template and the primers (lane 6) that were provided by Perkin Elmer. The PCR amplified product having the expected size (308 bp) was observed (lane 6). Molecular weight standards of λ-*Bst*EII and φX174-*Hae*III are shown in lanes 1 and 2, respectively. (B) The RT-PCR amplified product of hTAP from total RNA of T84 cells (lane 1) or from total RNA of NHBE cells (lane 2) was analyzed by staining the 1.4% agarose gel with ethidium bromide solution (0.5 μg/ml). Lane 3 corresponds to the DNA molecular weight standard, φX174-*Hae*III digest. (C) Nucleotide and predicted amino acid sequence of the RT-PCR amplified 220 bp product hTAP show its identity with bTAP. The predicted normal human TAP amino acid sequence is illustrated with 3-letter codes and an asterisk (termination) below the bTAP DNA sequence. The 5′ and 3′ primer sequences used for the RT-PCR experiment are underlined.

observed. Fig. 4 presents TEM of vertically cut thin sections of normal and CF cells 10 h after infection with *P. aeruginosa*. The cuts were made through the central region of the cells. In confirmation of results observed with horizontally cut thin

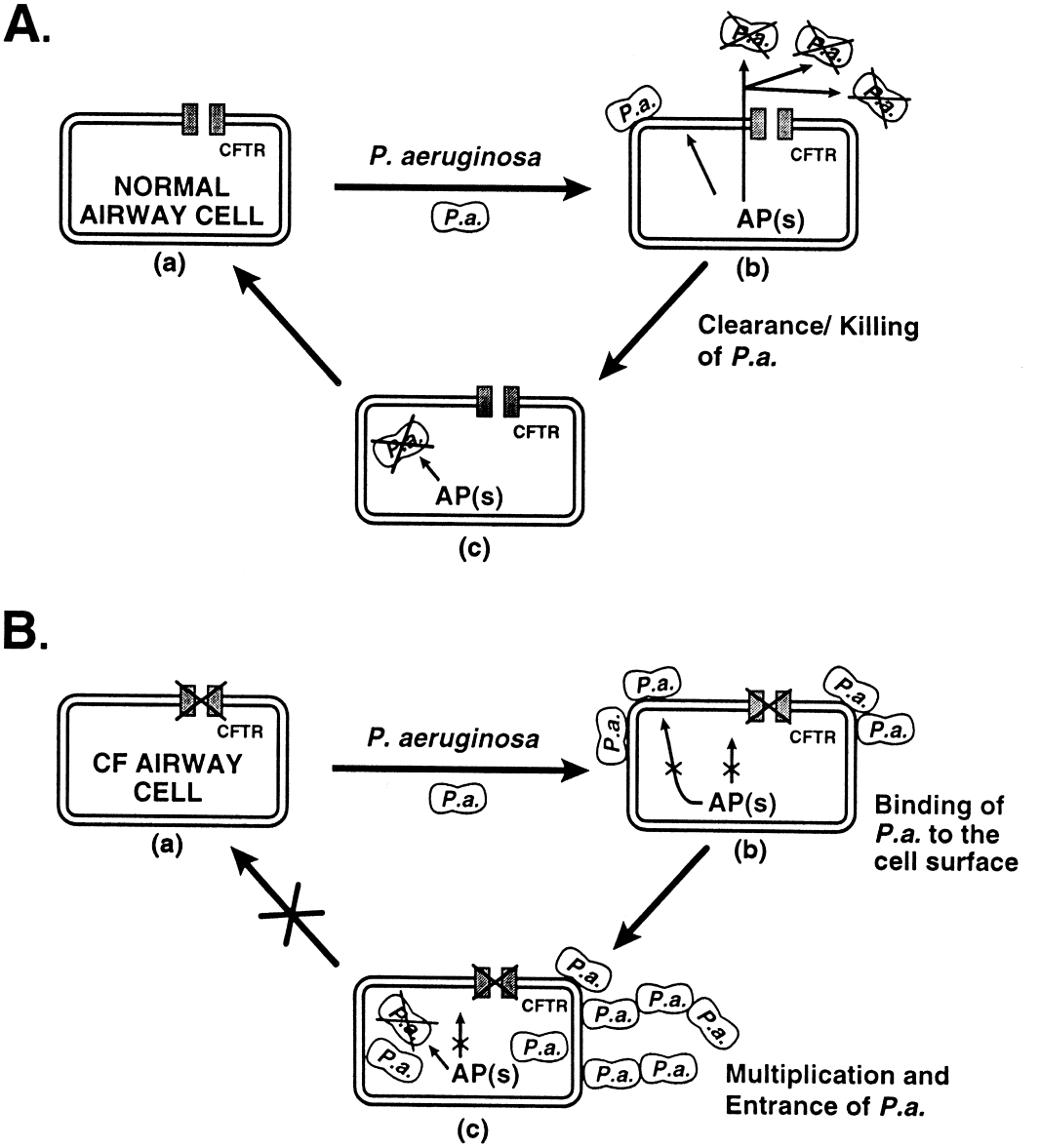

Fig. 6. Working model depicting how differently normal and CF epithelial cells interact with *P. aeruginosa*. (A) Normal cells (a) are shown preparing their first line of defense against invasion by *P. aeruginosa* (b). One or more antimicrobial peptides AP(s), perhaps hTAP, for destroying the microbial organism may be secreted by a mechanism dependent on the normal function(s) of CFTR (b) and may function also inside the cells against the entering *P. aeruginosa* (c). Consequently, infected cells finally clear the bacteria (a). (B) CF cells having defective CFTR (a) are infected with *P. aeruginosa*. Here, secretion of AP(s) which represent first line defense weapons, may be impaired or compromised (b). Thus, the efficiency of bacterial clearance by CF cells is much less than that of normal cells resulting in increased bacterial binding, multiplication, and entrance into cells (c), where killing by internally located AP(s) cannot keep pace with the rate of bacterial entry.

sections, these vertical cuts show no sign of bacteria bound to or within normal cells (top panel in green), but clearly reveal bacteria bound to and within CF cells (lower panel in magenta). Bacterial division also appears to occur within CF cells. It is important to note that bacteria are only about 1 μm in diameter whereas epithelial cells are 40–50 μm. Therefore, the finding that bacteria are observed in vertical sections cut centrally through CF cells is compelling evidence that the bacteria are inside the cell.

Although it is generally believed that *P. aeruginosa* do not enter epithelial cells, the extensive TEM studies reported here employing both horizontally and vertically cut thin sections, indicate that this organism does gain entry into ΔF508 CF cells in culture. The novel finding of bacteria inside CF cells may reflect either the cells' final line of defense in a 'last ditch stand' to fight back against an overwhelming microbial attack, or their surrender to the invading bacteria.

Recently, it was reported that cultured human airway epithelial cells expressing the ΔF508 allele of CFTR are defective in uptake of *P. aeruginosa* [9], and that only normal cells have the capacity to bind and allow entry of these infectious bacteria. Unfortunately, these investigators provided no electron micrographs as visual support for their conclusions and the 'normal' cells used were derived from ΔF508 homozygous cells that had been transfected with a plasmid containing wild-type CFTR. In a more recent study [10], although the preferential bactericidal action of normal airway epithelial cells relative to CF cells is noted, a NaCl concentration

(near 100 mM), in the normal physiological range for airway surface fluid [14,15], was implicated as key factor for the bactericidal action. Thus, in an experiment supported by limited visual examination of CF cells, the presence of *P. aeruginosa* was observed at a high but not a low Cl^- concentration [10]. However, in the extensive study reported here, where ~4000 CF cells were examined ultrastructurally after infection with *P. aeruginosa* at a low 'normal' NaCl concentration (104 mM), the capacity of these bacteria to bind and enter the diseased cells was unimpaired (Table 1, Fig. 2B–F). Significantly, in a recent erratum [19], it is reported that the Cl^- concentrations of 182 mM described by the above authors [10] to be characteristic of CF nasal epithelial fluid is not reproducibly observed. Therefore, the view [10] that CF cells cannot mount a strong defense against invading bacteria is because the salt concentration in the surrounding airway surface fluid is higher than normal requires further investigation.

In normal cells, the primary first-line defense against *P. aeruginosa* may involve secretion of one or more antimicrobial peptides. Significantly, we have shown by RT-PCR analysis (Fig. 5A) that human tracheal epithelial cells express an mRNA that corresponds closely to that of bovine TAP (bTAP), known to exhibit bactericidal activity against *P. aeruginosa* [20]. The expression of bTAP is reported to be specific to bovine airway mucosa, but not to several other tissues [21]. In humans, as in the bovine, the expression of the gene may be specific to the airway cells, as we have found it to be absent in the colon carcinoma cell line, T84 (Fig. 5B) where bactericidal activity is not necessary. The DNA sequence of the human tracheal epithelial antimicrobial peptide (hTAP) is identical to that of bTAP from Ala-9 to Arg-56 (Fig. 5C).

4. Conclusions

In summary, results of the present study using ultrastructural analysis by TEM clearly demonstrate that human tracheal epithelial cells containing wild-type CFTR interact differently with *P. aeruginosa* than ΔF508 CF cells, in which the bacteria bind and enter the diseased cells. Moreover, normal cells express the antimicrobial peptide hTAP, which is essentially identical to bTAP and is known to kill *P. aeruginosa*. This suggests a plausible working model (Fig. 6) to account, at least in part, for how normal tracheal cells may clear *P. aeruginosa*, whereas ΔF508 CF cells are unable to mount an effective defense. Thus, one or more antimicrobial peptides, AP(s), one of which may be hTAP, may play a CFTR-dependent role as part of a first line defense, functioning outside of, or if necessary inside of, tracheal epithelial cells. This role may be dramatically impaired or compromised in diseased (ΔF508) cells. Work reported here is potentially very relevant to the development of novel therapies to treat cystic fibrosis and many other lung diseases.

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References

[1] Cliff, C.H., Schomacher, R.A. and Frizzel, R.A. (1992) Am. J. Physiol. 262, C1154–C1160.
[2] Thomas, P.J., Shengagamurthi, P., Sondek, J., Hullihen, J.M. and Pedersen, P.L. (1992) J. Biol. Chem. 267, 5727–5730.
[3] Ko, Y.H., Thomas, P.J. and Pedersen, P.L. (1993) J. Biol. Chem. 268, 24330–24338.
[4] Ko, Y.H. and Pedersen, P.L. (1995) J. Biol. Chem. 270, 22093–22096.
[5] Hartman, J., Huang, Z., Rado, T.A., Peng, S., Jilling, T., Muccio, D.D. and Sorscher, E.J. (1992) J. Biol. Chem. 267, 6455–6458.
[6] Anderson, M.P., Berger, H.A., Rich, D.P., Gregory, R.J., Smith, A.E. and Welsh, M.J. (1991) Cell 67, 775–784.
[7] Saiman, L. and Prince, A. (1993) J. Clin. Invest. 92, 1875–1880.
[8] Imundo, L., Barasch, J., Prince, A. and Al-Awqati, Q. (1995) Proc. Natl. Acad. Sci. USA 92, 3019–3022.
[9] Pier, G.B., Grout, M., Zaidi, T.S., Olsen, J.C., Johnson, L.G., Yankaskas, J.R. and Goldberg, J.B. (1996) Science 271, 64–67.
[10] Smith, J.J., Travis, S.M., Greenberg, E.P. and Welsh, M.J. (1996) Cell 85, 229–236.
[11] Imundo, L., Barash, J., Prince, A. and Al-Awquati, Q. (1995) Proc. Natl. Acad. Sci. USA 92, 3019–3023.
[12] Giverson, R.T. and Demaree, R.E. Jr. (1995) Microsc. Res. Techn. 32, 246–254.
[13] Paton, G.P. (1995) J. Histochem. Cytochem. 43, 731–733.
[14] Ansorge, W., Sproat, B., Stegemann, J., Schwager, C. and Zenke, M. (1987) Nucl. Acids Res. 15, 4593–4602.
[15] Connel, C., Fung, S., Heiner, C., Bridgham, J., Chakerian, V., Heron, E., Jones, B., Menchen, S., Mordan, W., Raff, M., Recknor, M., Smith, L., Springer, J., Woo, S. and Hunkapillar, M. (1987) Biotechniques 5, 342–348.
[16] Middendorf, L.R., Bruce, J.C., Bruce, R.C., Eckles, R.D., Grone, D.L., Roemer, S.C., Sloniker, G.D., Steffens, D.L., Sutter, S.L., Brumbaugh, J.A. and Patonay, G. (1992) Electrophoresis 13, 487–494.
[17] Gilljam, H., Ellin, A. and Strandvick, B. (1989) J. Clin. Lab. Invest. 49, 121–124.
[18] Joris, L., Dab, I. and Quinton, P.M. (1993) Am. Rev. Respir. Dis. 148, 1633–1637.
[19] Smith, J.J., Travis, S.M., Greenberg, E.P. and Welsh, M.J. (1996) Cell 87 (Erratum).
[20] Diamond, G., Zasloff, M., Eck, H., Brasseur, M., Maloy, W.L. and Bevins, C.L. (1991) Proc. Natl. Acad. Sci. USA 88, 3952–3956.
[21] Diamond, G., Jones, D.E. and Bevins, C.L. (1993) Proc. Natl. Acad. Sci. USA 90, 4596–4600.