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Surfactant–ultrafine particle interactions: what we can learn from PM_{10} studies

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There is increased concern about the associations between particulate air pollution and human health. Inhaled and deposited particles play a crucial role in the aetiology of a range of pulmonary diseases. A variety of pulmonary diseases develop from the inhalation and deposition of pathogenic organisms or noxious particles (e.g. viruses, bacteria, spores, pollen, etc.). The inhalation of soot, burned tobacco and paper leads to common pulmonary diseases: chronic bronchitis and lung cancer.

It has been suggested that ultrafine particles might be taken up by cells, including by airway epithelial cells, through a process related to the surface forces exerted on them at the cell membrane–particle interfacial region.

Keywords: airways; surfactant; surface forces; primary defence barrier; particle–surfactant interaction; particle–cell interaction

1. Introduction

With each breath millions of particles enter the lungs, where they may land on the surface of the conducting airways or the alveoli in the gas-exchange region. Upon making contact with the wall (deposition), the processes of retention and clearance begin. These processes depend on many factors, including

(1) particle size, shape, solubility, surface chemistry and elastic properties of both the particles and the lung surfaces;

(2) the anatomical location of the deposition (alveoli, conducting airways), which is important for the route and distance of particle clearance;

(3) the histological structures the particles interact with at the site of deposition, including cells and the surfactant film at the air–liquid interface.

All these factors determine the fate of deposited particles and, hence, they are important for the therapeutic or pathogenic potential of the retained particles.

Clearance of particles deposited in the gas-exchange region is slow; it may last for months or years and involves phagocytosis by cells of the defence system, cell migration and, eventually, mucociliary transport. Particles deposited in the conducting airways, on the other hand, may generally be cleared within 24 h. However, data

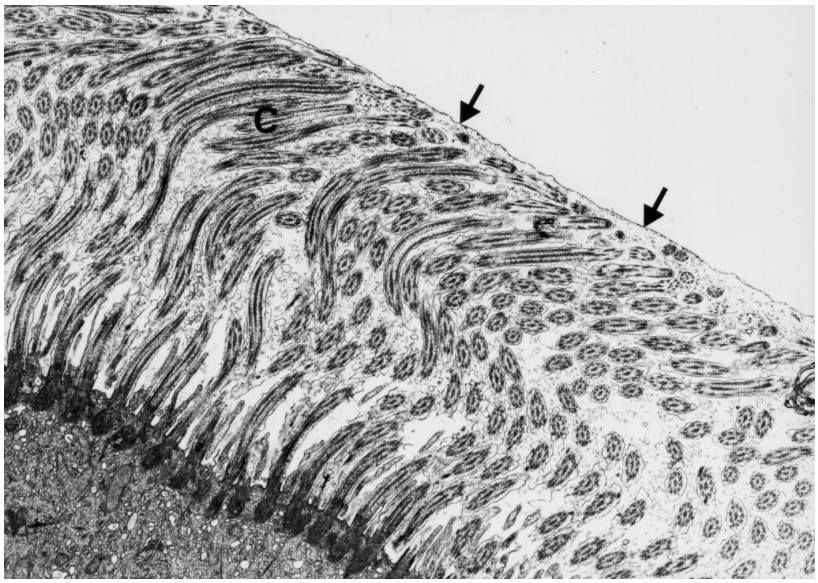

Figure 1. Transmission electron micrograph of tracheal epithelium of horse. It shows the apical part of the epithelium with cilia (C) in the extracellular aqueous liquid and a surfactant film at the air–liquid interface (arrows) (magnification 6.500×).

from the GSF–National Research Centre for Environment and Health in Munich (Scheuch *et al*. 1996) and from our laboratories (Geiser *et al*. 1990*a*) have shown that particles of 6 µm in diameter and smaller are not entirely cleared within this period. Inhalation studies in humans have shown that the fraction cleared within 24 h decreases with decreasing particle size from greater than 90% for particles greater than 6 µm in diameter to less than 30% for particles less than 1 µm in diameter (Scheuch *et al*. 1996).

There are reports on ultrafine particles (less than 0.1 µm in diameter) which show that these tiny particles are more toxic than larger particles (Oberdörster *et al*. 1994). The enhanced toxicity may be related to their greater surface area relative to the particle mass, and so may depend on the provision of more sites to interact with cell membranes and a greater capacity to adsorb and transport toxic substances such as acids (Chen *et al*. 1992). Acidic dusts are powerful irritants and stimulate mucus secretion. A single intratracheal instillation of a sample of airborne dust collected in Ottawa (3–5 µm mass median aerodynamic diameter, 1.9 µm geometric standard deviation) was found to cause a marked increase in thickness of the mucus layer from 2 to greater than 30 µm within *ca.* 5 min (Green *et al*. 1995).

Could the adverse health effects of small particles in the lungs be due to their prolonged retention time? With regards to the particles deposited on the airway wall and their clearance, the air–liquid interface is of high significance. Like the alveolar surfaces, the airway surfaces are also covered by a surfactant film (figure 1). This surface layer consists of phospholipids and specific proteins. It is surface active and, hence, results in a reduction of the surface tension at the airway surface (Geiser *et al*. 2000; Gehr *et al*. 1990; Schürch *et al*. 1990).

The alveolar surfactant film stabilizes the gas-exchange area of the lung by reducing the surface tension at the air–liquid interface. In contrast, the mechanical functions

of the airway surfactant are not well established. One suggested function is particle transport from the alveoli to the ciliated airways by a surface tension gradient (Horn & Davis 1975; Podgorski & Gradon 1993), another property of airway surfactant is particle displacement toward the epithelium. In the following we will focus on this latter experimentally established property of airway surfactant.

After their deposition on the surfactant film of the airway wall, particles are wetted and displaced toward the epithelium by the surfactant film during the retention process. This translocation of the particles by capillary forces is one of the mechanical properties of the surfactant film (figure 2). *In vitro* experiments using a modified Langmuir–Wilhelmy balance have demonstrated that the extent of particle immersion depends on the surface tension of the surfactant film (e.g. dipalmitoyl phosphatidyl-choline (DPPC) of lipid extract surfactant) for a particular particle shape and surface chemistry. The lower the surface tension (or the higher the surface pressure), the greater is the immersion of the particles into the aqueous phase (figure 3) (Gehr *et al*. 1990; Schürch *et al*. 1990). This immersion, however, neither depends on the particle density nor on substrate chemistry. This has been clearly demonstrated by choosing a liquid substrate whose density is higher than that of the particles.

Figure 4*a* shows polymethylmethacrylate particles of different sizes retained on a guinea pig trachea. Smaller particles are totally submerged into the aqueous phase below the osmiophilic surfactant film. Larger particles show various degrees of displacement into the liquid phase.

The rheological characteristics of the aqueous liquid phase, which partly consists of mucus, are well matched with the characteristics of the ciliary propulsion system, and facilitate mucociliary transportation. It depends on the interplay between three components, the cilia, the periciliary (less viscous) fluid, and the mucus, with the surfactant film at the liquid–air interface. The mucus, which is not a continuous layer, but rather exists in flakes or sheets, is not an impermeable barrier for particles deposited at the air–wall interface. The glycoproteins of mucus are most important in providing the appropriate levels of elasticity, viscosity and cohesiveness for mucus flakes and sheets to be optimally propelled (Silberberg 1983).

Particles have been thought to stick to the viscous and ‘sticky’ mucus. However, they do not just stick to it, they are displaced by surface forces exerted on them by the surfactant film and are ‘engulfed’ by the mucus layer. It has been demonstrated by experiments with the Langmuir–Wilhelmy balance that, for particle immersion, the aqueous substrate on the airway wall may be mimicked *in vitro* by a viscous Newtonian fluid whose density is higher than that of the particles, provided the surface tension of the surfactant film in the balance is the same as that at the airway wall–air surface.

The *in vitro* experiment also showed that smaller particles are wetted by the substrate to a substantially greater extent than larger ones (figure 4*b*). One may also conclude from these experiments that small particles are more effectively displaced by a fluid phase covered with a surfactant film than larger particles, and that this effect is not related to the thickness of the aqueous liquid phase. The exact displacement mechanisms, especially the initial wetting process of particles of differing sizes, are not yet understood and require further experiments.

Explanations for the difference in displacement according to particle size are offered by the theory of surface thermodynamics, dealing not just with dividing interfaces

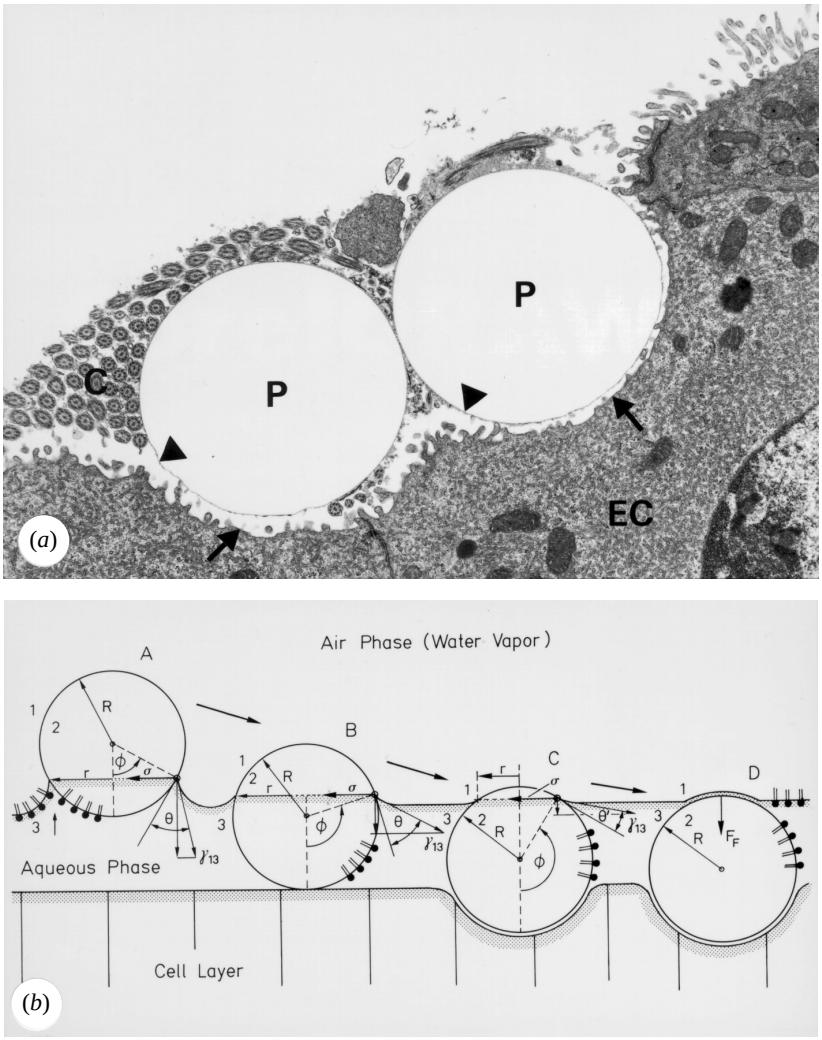

Figure 2. Particle displacement. (*a*) Transmission electron micrograph of the airway epithelium of a hamster. It shows two polystyrene particles (P) of a test aerosol, 6 µm in diameter, deposited on the airway wall and subsequently displaced into the aqueous liquid lining layer. Note the indentations (arrows) into the epithelial cells (EC) and the cilia (C) surrounding the particles. Arrow heads point to surfactant film (magnification 9.000×; see Geiser *et al.* (1990*b*)). (*b*) Schematic of the immersion of a particle. In addition to surface tension, line tension is considered. Line tension is the one-dimensional analogue of surface tension or the excess free energy density associated with the linear phase where the phases vapour, fluid+film and solid join. A, situation immediately after deposition. B, particle is further displaced. C, surface tension in conjunction with line tension promotes further particle displacement. D, particle is below surfactant film, which may be considered as an elastic skin keeping the particle submerged. θ is the contact angle, R the particle radius, r the radius of the three-phase line, σ the line tension, γ the surface tension, and ϕ indicates position of the three-phase line (Gehr *et al.* 1996).

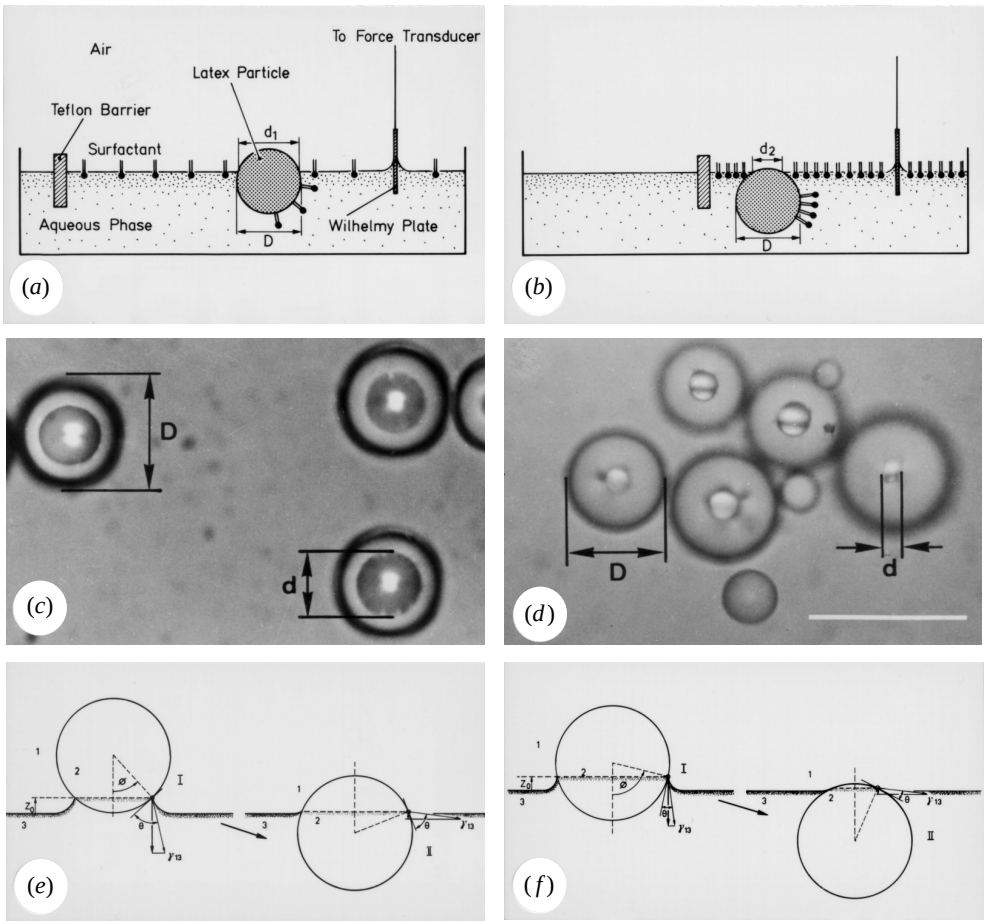

Figure 3. *In vitro* experiment with particles in a Langmuir–Wilhelmy surface balance. Two mechanically enforced stationary states of polystyrene spheres. Schematic of sphere that had been deposited on a DPPC monolayer at higher (*a*) and at lower (*b*) surface tension. (*c*) and (*d*) show polystyrene particles placed onto a DPPC monolayer on saline–sucrose subphase; the density of the subphase was higher than the density of the particles. The surface tension was higher in (*c*) than in (*d*). (*e*) and (*f*) are schematic diagrams of the two stationary states of particles. Higher contact angle (*e*); lower contact angle (*f*), characteristic for greater wettability by the fluid phase (Gehr *et al*. 1996).

and the related surface tensions, but also with dividing lines and the related line tensions (Geiser *et al*. 2000). Detailed discussion of these forces would, however, be beyond the scope of the present paper.

After deposition, particle solubility, shape, size and the surface properties of the particles largely determine their fate and, hence, also their residence time in the respiratory tract, with consequences for the generation of lung disease. As shown by the model studies outlined above, wetting and displacement of particles at the air–aqueous substrate interface depend on surface forces and also, probably, on line tension effects. These forces also determine whether or not the particle is brought into close contact with the epithelial cells and, in particular, with cells of the defence

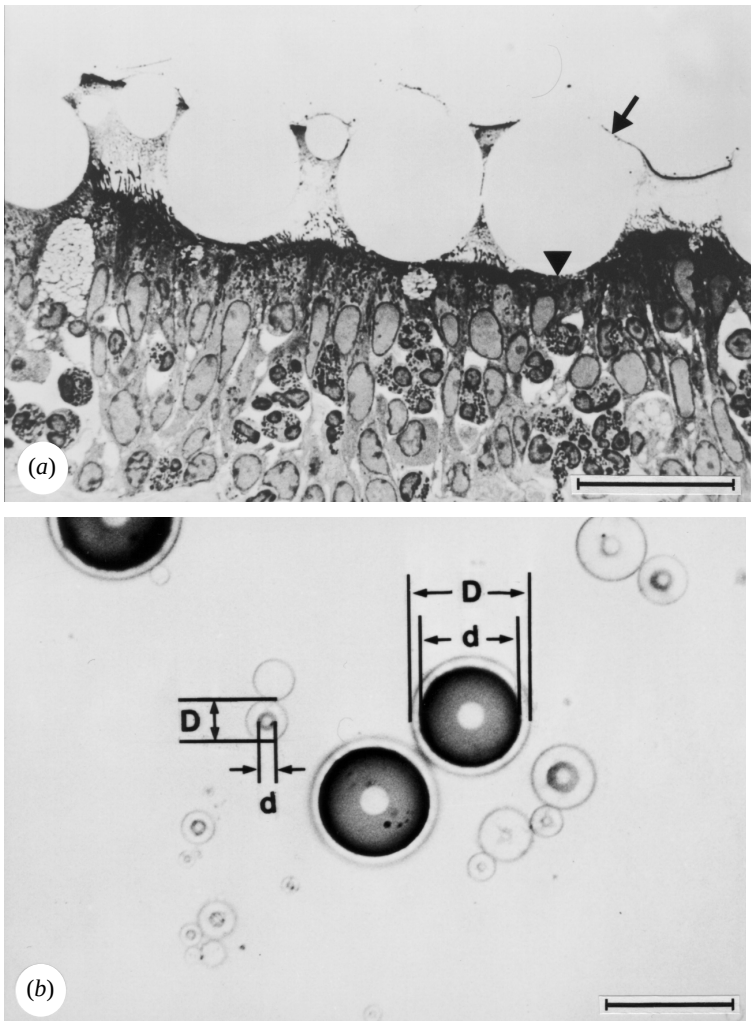

Figure 4. Displacement of particles of different sizes. (*a*) Light micrograph of tracheal wall of guinea pig, which had been exposed to a polydispersed aerosol of polymethylmethacrylate particles. The particles are submerged beneath an osmiophilic film (arrow) and indent the underlying epithelium (arrow head). Bar equals 30 µm (Gehr *et al*. 1996). (*b*) Light micrograph of polymethylmethacrylate particles of different size on a DPPC film, supported by a saline–sucrose subphase. D is the total diameter, d is the diameter of the segment exposed to air. The ratio d/D for the small particles is much smaller than that for the larger particles. This indicates greater immersion into the subphase of the small particles compared with the large particles. Bar equals 50 µm (Geiser *et al*. 2000).

system, like macrophages on the epithelial layer, or with dendritic cells. Dendritic cells are located at the base of the epithelium and reach up to the tight junctions between the epithelial cells with long fine cytoplasmic processes. Surface and line tension forces depend on the interfacial properties of the interacting systems, including the particles themselves and the surrounding aqueous medium, with the interfacial film between medium and particle. Particles with a low surface free energy, such as

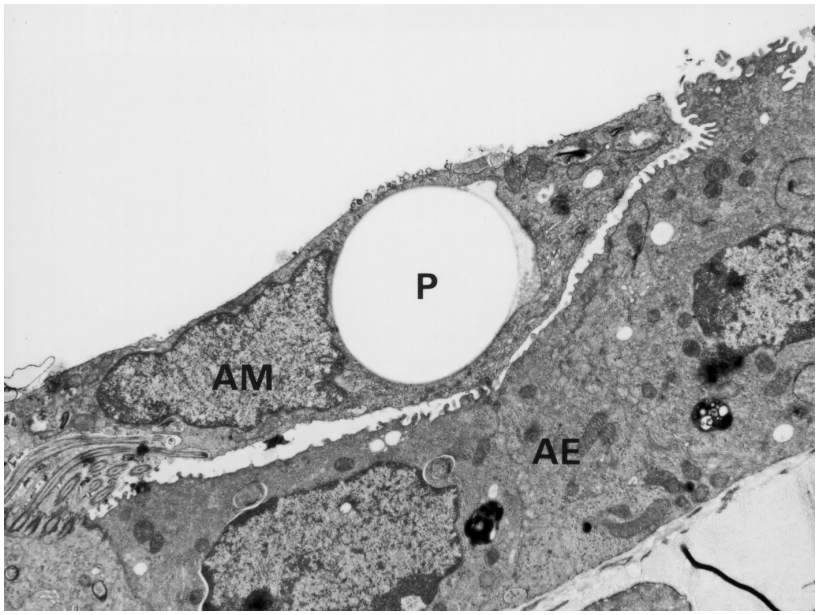

Figure 5. Transmission electron micrograph of airway macrophage (AM) which had phagocytized a polystyrene particle (P), closely associated with airway epithelium (AE) (magnification 6.000×; see Geiser *et al*. (1990*b*)).

Teflon, will generally be immersed less readily than high-energy particles, such as glass. Hydrated particles, such as bacteria, with a relatively high surface free energy would be substantially more readily wetted and displaced than hydrophobic particles.

The forces associated with the free energy of interfaces and dividing lines might also contribute substantially to particle–cell interactions. These interactions are considered non-specific, in contrast with specific receptor–ligand interactions. Thus, non-specific interactions may contribute to the uptake of particles by cells such as epithelial cells that do not generally function as phagocytic cells. These findings may have implications for particle pathogenicity and persistence of small particles in the submicrometre range (ultrafine particles, nanoparticles) (Oberdörster *et al*. 1994).

The process of dislocation of particles into the aqueous liquid phase is important, as only particles in proximity to the cells of the defence system can interact with these cells, that is with macrophages and eventually with dendritic cells and epithelial cells. We postulate that the initial step of retention, the particle–surfactant film interaction, determines whether the particles are carried away free or in macrophages (figure 5), i.e. by professional phagocytes via the airways (mucociliary clearance), or whether they are transported into the tissue via dendritic cells, i.e. professional antigen-presenting cells. These cells may process the particles and carry them to the specific immunological defence system, present them to T-lymphocytes and eventually initiate an immune reaction (figure 6). Because of this function and the crucial role they play in the pulmonary defence system, dendritic cells in the lungs are called 'sentinels' of the pulmonary immune system (McWilliam *et al*. 2000; Holt & Schon-Hegrad 1987).

In the primary defence against noxious particles, the macrophages play the leading role. They engulf as much material as possible and produce and secrete media-

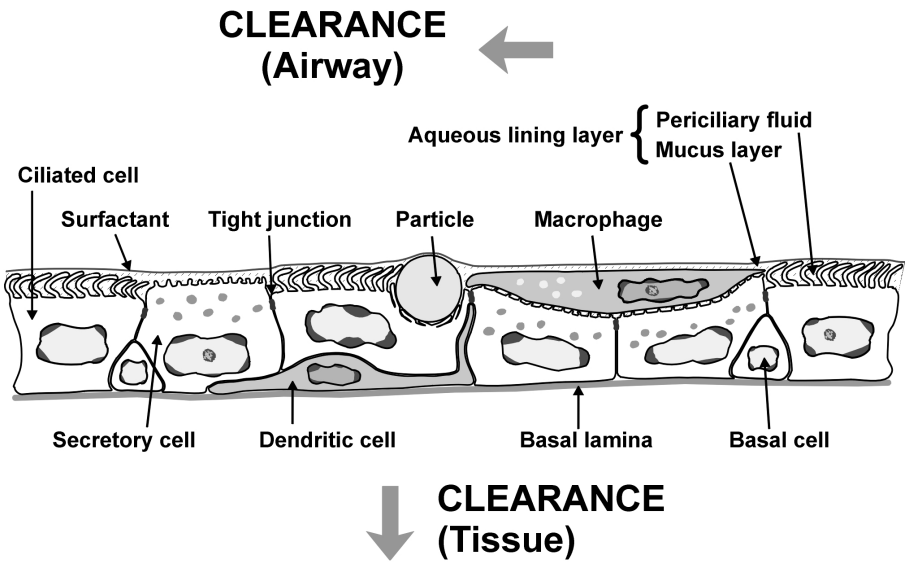

Figure 6. Schematic of airway epithelium showing the postulated close vicinity of a retained particle with epithelial cells, airway macrophages and dendritic cells (McWilliam *et al*. 2000).

tors, causing an inflammatory or immune reaction. In order to be able to trigger an immune reaction, T-lymphocytes must first be activated. As T-lymphocytes cannot react independently to an antigen, they need the help of accessory cells absorbing and processing the antigen and presenting it to them in a proper form.

Dendritic cells build a three-dimensional network in the airways, and seem to stay in contact with each other via long cytoplasmic processes reaching out between the epithelial cells and oriented parallel to the surface of the airway epithelium (figure 7*a*). The body of the cells is situated at the epithelial basis but on the epithelial side of the basal membrane. By means of cytoplasmic processes, dendritic cells push through inter-epithelial spaces perpendicular to the epithelial surface, almost reaching the airway lumen and separated from it only by the tight junctions (figure 7*b*) (McWilliam *et al*. 2000; Holt *et al*. 1990). Hence, they are in close association with particulate antigens and airway macrophages, which are in the aqueous layer below the surfactant (figure 6).

In contrast to the macrophages, which engulf as much material as possible for efficient clearance, dendritic cells probably take up only as much antigen material as necessary to stimulate an immune reaction when presented to the T-cells.

The mechanisms by which dendritic cells reach the particles or how the particles get to the cells have not yet been determined. On the one hand, particles phagocytized by macrophages are probably carried out of the lungs by mucociliary transport via airways. Particles engulfed by dendritic cells, on the other hand, are transported into the tissue, from where they reach the lymph nodes via the lymphatic drainage system (figure 6) (McWilliam *et al*. 2000; Gehr *et al*. 1996). However, a cooperation of the dendritic cells with macrophages on the luminal side of the epithelium and with the epithelial cells themselves may be a possible mechanism for the trans-epithelial transport of particles.

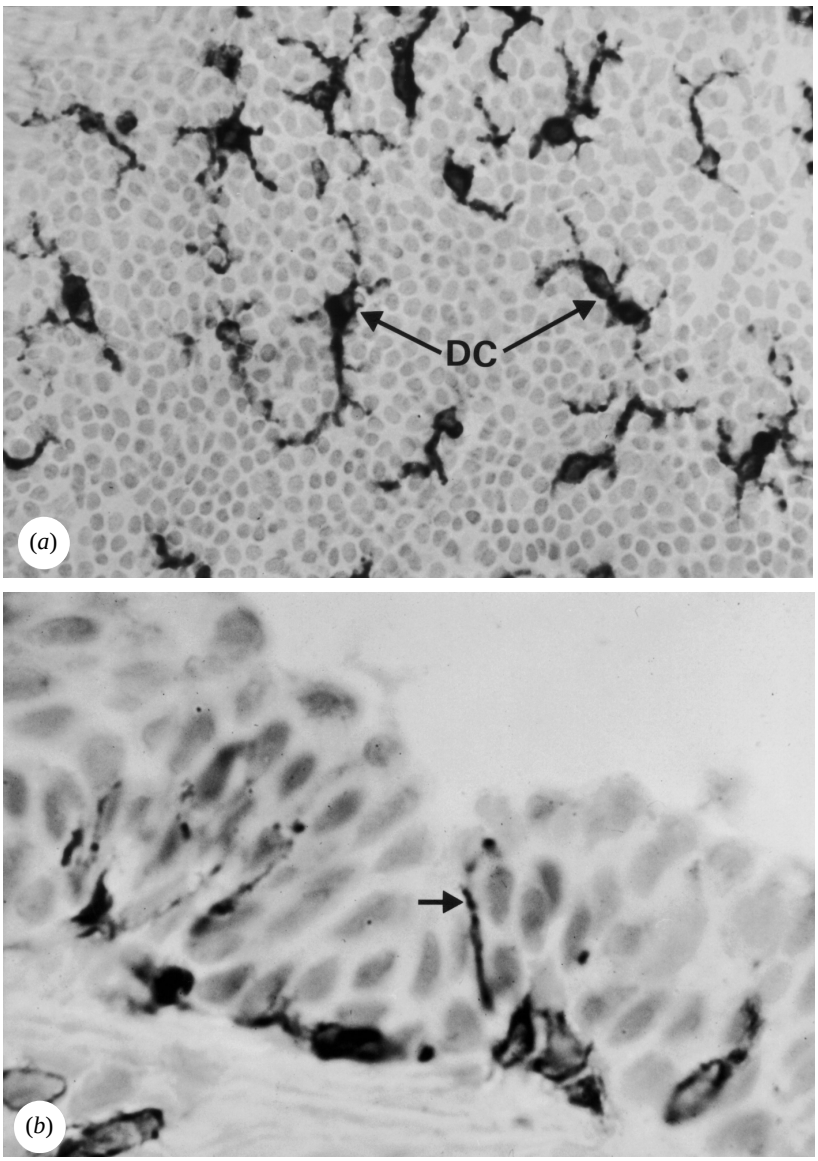

Figure 7. Light micrographs of dendritic cells (DCs), stained with Ox6 against rat MHC class II antigen, in rat tracheal epithelium. (*a*) Horizontal section through the epithelium, showing the DCs eventually communicating with each other with their long cytoplasmic processes (magnification 500×). (*b*) Section perpendicular to epithelial surface, showing the cell bodies at the base of the epithelium and long cytoplasmic processes reaching up close to the airway lumen (arrow) (magnification 800×). (Courtesy of P. G. Holt, Institute for Child Health Research, University of Western Australia, Perth, Western Australia).

In vitro experiments have indicated that dendritic cells are less capable of phagocytosis than macrophages, but the phagocytic capacity of dendritic cells is much higher than expected, even though this is only true during their immature stage (Kiama *et al*. 1999) (figure 8). While the cells mature, their capability of antigen

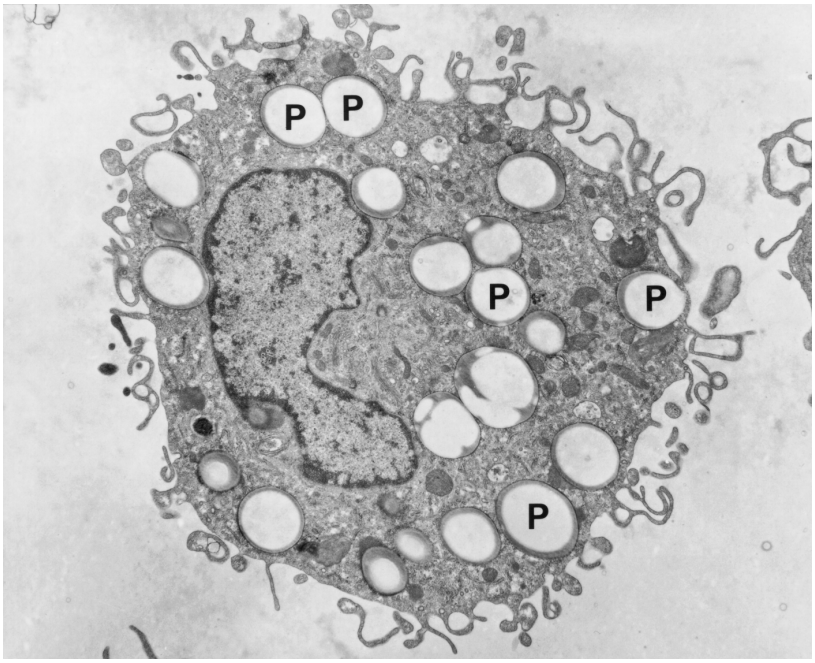

Figure 8. Transmission electron micrograph of dendritic cell in culture, where it was exposed to 1.5 µm polystyrene particles (P) (magnification 11.000×). (Specimen courtesy of L. P. Nicod, Cantonal Hospital, University of Geneva, Geneva; micrograph courtesy of S. G. Kiama, Institute of Anatomy, University of Bern, Bern, Switzerland).

presentation is developed and the capability of phagocytosis disappears almost completely. During this process the dendritic cells leave the epithelium and migrate to the lymph nodes (McWilliam *et al*. 2000; Kiama *et al*. 1999).

The displacement of particles deposited on the surfactant film by surface and possibly line tension forces exerted on them by this film is probably the initial step of a complex cascade of defence processes in the lungs. Surfactant, or more precisely its film, may, therefore, be called a primary defence barrier. Through opsonization by surfactant or surfactant components during displacement by wetting, particles are probably rendered less toxic and more attractive to phagocytic cells. It is proposed that the opsonization helps guiding particles along that clearance pathway which is most beneficial for our health, namely up the airways and out of the organism, or into the tissue to be presented to the specific defence system (figure 6) (Gehr *et al*. 1996).

It is of great importance and remains to be determined whether ultrafine, submicron particles experience the same fate as the micrometric size particles considered above. It has already been suggested that ultrafine particles might be taken up by cells, including by airway epithelial cells, through a process related to the surface forces exerted on them at the cell membrane–particle interfacial region.

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Discussion

R. L. Maynard (*Department of Health, Skipton House, 80 London Road, London*). You pointed out that as a surfactant film contracts and its surface tension falls, particles are drawn through the surface. Surfactants have a range of roles including stabilizing alveoli of different sizes and reducing the movement of water into alveoli: these effects are dependent on the low and variable surface tension of the surfactant film. Is the effect you described an undesirable side-effect of these functions?

P. Gehr. I wouldn't call the displacement of particles by surface forces exerted on them by surfactants a side-effect. It is just another effect which was unknown

until particle retention was investigated, i.e. '[s]ince the second half of the eighties'. This effect is more pronounced in the alveoli since the surface tension becomes very low (less than 1 nN m) at expiration. However, as recently found by my colleagues Marianne Geiser and Samuel Schuerck, even with the much higher surface tension in the airways (but lower than that of mucus and other biopolymers), all particles smaller than 10 μm were found to be displaced.

C. Kim (*National Health and Environmental Effects Research Laboratory, US EPA, Research Triangle Park, NC, USA*). Particles deposited in the airways are cleared out of the lung by mucociliary escalator.

It is believed that mucociliary transport is efficient only if particles are positioned on the mucus layer. If particles are forced to sink down through the mucus layer, or they are landed in the areas where mucus layer is absent, those particles may not be cleaned by mucociliary escalator. Do you have any suggestions concerning how much of the particles deposited in the airways may be found on the epithelial surface?

P. Gehr. But these particles may eventually be cleared by ciliary activity (as we have observed but which needs to be defined). The displacement may get particles into (not through) the mucus; that is, the mucus will act as a containing space for transportation of the particles.

The number of particles found on the epithelial surface probably depends on the size of the particles, it might be most of them or only a few. We only have observations of this process, this needs to be studied in more detail. From studies of the GSF (W. Stahlhofen, G. Scheuch and J. Heyeder) in Munich it is known that the smaller the deposited particles the smaller the fast clearing fraction is.

H. Rees (*Therapeutics and Toxicology Centre, UWCM Academic Centre, Llandough Hospital, Cardiff, UK*). You presented information on the interactions between fine polystyrene particles and respiratory epithelium. Have you studied the behaviour of other materials? Have you found that polytetrafluorethylene (Teflon) particles and asbestos fibres behave much like polystyrene, and that the behaviour of different materials can be predicated by size?

P. Gehr. Our inhalation studies with different types of fine particles like polystyrene, Teflon, puff ball spores and glass fibres showed that they are all displaced into the liquid layer by surface forces if deposited on the surfactant film at the air–liquid interface. The displacement process depends on the size rather than on the shape and type of the particles, i.e. all inhalable particles will be displaced. I would predict that asbestos fibres also behave in the same way.