Simvastatin inhibits smoke-induced airway epithelial injury: implications for COPD therapy

To the Editor:

I read with interest the report by DAVIS *et al.* [1] on epithelial injury-shedding *in vivo* caused by tobacco smoke. Their model involves airways equipped with human-like subepithelial microvessels carrying systemic blood. By contrast, mice lack a bronchial microcirculation. Severe bronchial epithelial damage is induced with widespread cell loss from an apparently intact epithelial basement membrane. By weeklong pre-treatment with simvastatin, the smoke-induced epithelial derangement and associated inflammation are largely prevented. Although a high dose was used, the drug effect is impressive. The authors apparently excluded: 1) involvement of oxidative stress, as reflected by haem oxidase-1, in the smoke-induced epithelial desquamation; and 2) mevalonate-dependent mechanisms in the ''therapeutic'' effect of simvastatin. They discuss the possibility that simvastatin inhibits granulocyte-induced epithelial injury but also mention that the drug may target epithelial cells directly. Most previously reported anti-inflammatory actions of simvastatin are dependent on the mevalonate pathway, making the authors' observations stand out. Incidentally, mevalonate independence is compatible with direct epithelial effects of simvastatin involving inhibition of interferon regulatory factor 3 [2]. By this mechanism, simvastatin reduces epithelial production of select cytokines/mediators, but there is no known link with epithelial cytoprotection. Perhaps the bronchoalveolar fluid collected by DAVIS *et al.* [1] can provide further information on released epithelial factors? For example, to what extent did the drug preserve epithelial innate immunity function?

During recent decades the epithelial field has, to a large extent, been addressed by cell culture approaches, hiding the in situ epithelium's interaction opportunities. Particularly important is the supply of bioactive molecules and cells from a profuse subepithelial microcirculation. The *in vivo* approach taken by DAVIS *et al.* [1] is, therefore, commendable. However, they fail to mention that mere loss of epithelial lining cells, without inflammatory insults, will initiate a series of significant acute, as well as more sustained, effects *in vivo* [3–6]. A discussion of effects of epithelial injury-repair processes and their sequelae is justified in view of the dramatic denudation produced in the *in vivo* study by DAVIS *et al.* [1]. This comment is strengthened by potential involvement of epithelial shedding-induced effects in inflammatory and remodelling features of asthma and chronic obstructive pulmonary disease (COPD) [3, 6–8]. Indeed, the damaged epithelium illustrated by DAVIS *et al.* [1] may be more reminiscent of asthmatic than COPD bronchi, perhaps making their model one of severe asthma overlapping with COPD.

My arguments are underpinned by original work carried out by Ingrid and Jonas Erjefält and others in the mid-1990s. Unfortunately, there appears to have been limited development since then regarding epithelial shedding-restitution events and sequelae *in vivo* in human-like airways [7, 8]. I acknowledge that leading laboratories have advanced asthma and COPD research immensely with sophisticated culture methods revealing intriguing differences between health and disease, and I apologise for not reviewing in vitro experiments here. I also appreciate that the contribution from the microcirculation is like opening a Pandora's Box, potentially blurring favoured molecular in vitro mechanisms. Hence, in view of prevailing priorities in biological–medical research, the aspects discussed here are not frequently entertained in the current literature. Yet, in a discussion of the *in vivo* findings of DAVIS *et al.* [1], they may merit a revisit.

If sloughing of epithelium leaves an intact basement membrane, epithelial repair starts immediately and proceeds rapidly (over a damaged basement membrane, repair is severely delayed as it may be in cell culture). All cells bordering the denuded area *in vivo* participate in the repair [4]. Thus, ciliated and secretory cells, once considered end-differentiated cells, internalise their cilia and lose their secretory granules, respectively; this occurs within few minutes after denudation, as these cells promptly transform into flattened repair cells that speedily (several microns per minute) migrate to produce a new cell cover [4]. There is thus little need for recruitment of epithelial progenitor cells from the circulation. The speed with which patchy denuded areas can be covered *in vivo* may explain, in part, why increased bronchial absorption permeability has been so difficult to demonstrate in asthma and COPD patients [3, 5, 7].

The basement membrane appears to be unharmed in the study by DAVIS *et al.* [1], yet the authors do not report signs of epithelial repair in their sections of bronchial tissue. A problem may be that sectioning itself causes denudation in airways with a fragile epithelium [8]. Hence, whole-mount tissue preparation, rather than sectioning, is probably a preferable method for demonstrating sites of epithelial damage repair [9]. Observations of whole mounts have revealed that the injury caused by inhaled noxious agents is exceedingly patchy. This is so even when it is ensured that the mucosal surface area is equally exposed *in vivo* to a culprit challenge [7, 9]. In an inflamed airway, actual patches of epithelial sloughing thus occupied $<1\%$ of the surface area. However, this limited injury was seen as $\geqslant 20\%$ frank denudation in cryostat sections; no denudation occurred in sections of uninflamed airways [9]. It should thus be of interest to see the distribution of epithelial sloughing induced by tobacco smoke in whole-mount preparations of the exposed mucosal surface.

Denudation occurs without any bleeding [3]. However, signals from the denudation site go to the profuse subepithelial microcirculation, causing prompt and lasting extravasation of bulk plasma from post-capillary venules [10, 11]. Similarly, extravasation of leukocytes is also prominent. Activated neutrophils may dominate, but in an already eosinophilic mucosa, the eosinophils join in and become activated [11]. Eosinophils degranulate by the disease-relevant mode of primary cytolysis [3, 6] and the resulting free eosinophil granules abound at patchy sites of epithelial injury/repair [12]. The early phases of epithelial restitution after denudation thus occur in a dynamic fibrin–fibronectin gel replete with activated granulocytes (epithelial cells in the area of interest apparently do not produce fibrin–fibronectin [10]). Epithelial hotspots of shedding restitution processes also send out signals causing hypersecretion from surrounding mucosal goblet cells (fig. 1) [4].

As soon as the denuded basement membrane has become well covered by a layer of migrating repair cells (note that these undifferentiated cells may mistakenly be reported as normal basal cells), the leukocyte-rich plasma protein-derived gel ceases to be fed from the microcirculation. It becomes degraded and is shed into the airway lumen. A granulocytic inflammatory picture, involving deranged epithelium, agrees with features of severe states of asthma and COPD. An inflammatory exudate, probably produced in part by the events discussed above, also correlates well with severity of asthma and COPD [13]. Indeed, HOGG *et al.* [14] reported that the presence of an inflammatory exudate best correlated with the severity of COPD. In summary, it is possible that injury repair processes themselves evoke part of the inflammatory responses to the smoke-induced epithelial injury [1]. The inhibition of this inflammatory response by simvastatin [1] would then reflect epithelial cytoprotection as a primary drug action.

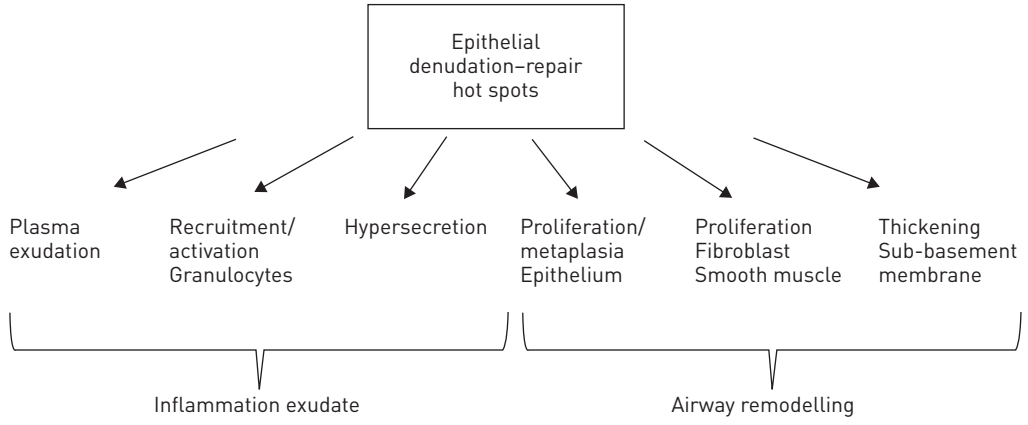

FIGURE 1 When airway epithelial cells are lost, exposing a denuded basement membrane, there are immediate responses from the profuse subepithelial microcirculation as well as from the neighbouring epithelium: all types of cells of the pseudostratified epithelium, bordering the denuded area, dedifferentiate to become rapidly migrating, flattened repair cells. This early-phase repair occurs in a microcirculation-produced, dynamic, granulocyte-rich, fibrin–fibronectin gel covering the basement membrane. Goblet cells in an area extending far beyond the repair site secrete extensively. When repair epithelium covers the naked basement membrane, the supply of proteins and leukocytes from the microcirculation ceases, the gel cover resolves and is shed, contributing to an inflammatory exudate. There is then a phase of epithelial metaplasia before the epithelium, if not further disturbed, eventually becomes normally differentiated. In addition, the repair evokes proliferation of subepithelial fibroblasts and smooth muscle cells, and there may be thickening of the sub-basement membrane. Thus, exaggerated epithelial shedding–repair events alone may contribute to inflammatory and remodelling features of asthma and chronic obstructive pulmonary disease.

DAVIS *et al.* [1] did not examine, nor did they discuss, effects on airway remodelling in their epithelial injury model. However, epithelial denudation repair involves a significant second phase with proliferation, metaplasia and differentiation of the epithelial lining cells. Epithelial metaplasia may characterise COPD bronchi but little evidence is really available. The epithelial restitution process further induces proliferation of subepithelial fibroblast and smooth muscle cells [4]. In addition, by repeated shedding restitution events, the epithelial sub-basement membrane may thicken [5]. However, this feature is not typical in COPD; moreover, it can be produced, along with several other remodelling features in asthma, by noninflammatory bronchoconstriction [15]. The structural proliferation changes associated with epithelial repair outlast responses such as inflammatory cell recruitment/activation and plasma exudation [4, 11]. This observation is of interest because severe asthma can exhibit epithelial–mucosal histopathology without ''inflammation'' [15]. Even more interesting perhaps, Creola bodies (clusters of desquamated epithelial cells) in aspirated sputum, independent of other factors, were reported to predict development of asthma in children [16]. As observed by NAYLOR [17], Creola bodies in sputum have also been significantly associated with exacerbations of asthma.

The epithelium holds the stage in current asthma/COPD research. The focus is on roles of epithelial cells as drivers of immunopathogenic mechanisms. This well-justified interest in molecular capacities of the strategically located epithelium has probably eclipsed a primary interest in the shedding–restitution aspects discussed above. Perhaps the predominance of cell culture studies over more demanding, but relevant, *in vivo* approaches has contributed to this balance. Further *in vivo* discoveries of mechanisms and pharmacological control of epithelial shedding and its sequelae are needed, I think, both to validate current notions and to develop novel research paradigms in this field of interest. The *in vivo* approach by DAVIS *et al.* [1] may turn out to be instrumental for therapeutic developments. There is an unmet medical need for ''cytoprotective'' drugs that reduce occurrence of epithelial injury in diseased bronchi.

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There is an unmet medical need for cytoprotective drugs that reduce epithelial injury occurrence in diseased bronchi http://ow.ly/qhMn7

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From the author:

Life is short, and the art is long

– Hippocrates

We read with interest C. Persson's letter to the editor. It brings to mind the above quote, as every problem or observation has many potential vantage points. Thus, one's work is never truly finished. In the age of molecular biology, genetics, bioinformatics and systems biology the focus of investigators has naturally shifted to reflect these advances. However, some of the most outstanding discoveries are rooted in earlier observations using *ex vivo* and *in vivo* models of disease.

The commentary by C. Persson is interesting, highly relevant and contains excellent ideas about potential statin epithelial cytoprotective mechanisms. The perspective regarding microcirculation in the rat model, unlike in mice that lack bronchial circulation, is particularly noteworthy. Therefore, on behalf of my co-authors, we appreciate the ideas put forth by C. Persson. We will attempt to address some of the questions raised; and at the end offer a few questions of our own regarding future research directions.

In our study [1], we showed that simvastatin has potent anti-inflammatory effects and prevents tobacco smoke-induced bronchial epithelial denudation. However, our experimental design did not allow us to draw firm conclusions regarding the mechanism(s) underlying these anti-inflammatory and cytoprotective effects. Importantly, we did not assess whether simvastatin's beneficial effects were mevalonate-dependent. Albeit both 1) total cholesterol and 2) small GTPase (Rho and Ras) expression and intracellular localisation did not change (in whole lung homogenate), this does not necessarily indicate mevalonate-independent statin effects.

One potential mechanism for the cytoprotective effect of statins is the induction of pro-resolution and anti-inflammatory factors at the mucosal level. The pro-resolving mediator 15-epi-lipoxin A_4 (15-epi-LXA_4) is important in the resolution of tissue inflammation. Simvastatin and lovastatin both increase the production of 15-epi-LXA_4 in activated human airway epithelial cells [2]. Lovastatin also decreases total and neutrophilic acute inflammation in airway mucosa by increasing the production of 15-epi-LXA_4 *in vivo* [2]. This is likely one of several mechanisms whereby statins protect the airway epithelium, *i.e.* by inducing endogenous pro-resolving mediators during injurious leukocyte–airway epithelial interactions.

C. Persson mentioned that the loss of epithelium may lead to airway inflammation without an inflammatory insult. In our model, smoke exposure induced an acute neutrophilic-predominant inflammatory response and epithelial sloughing throughout the rat bronchial tree. Pretreatment with simvastatin significantly mitigated both of these pathological changes. Whether simvastatin directly prevented epithelial injury due to local cytoprotective effects, or whether simvastatin did this indirectly by reducing leukocyte influx into the airways is not known in our experiments.

In a recent study, JUNCADELLA *et al.* [3] reported that Rac1-dependent defective engulfment of apoptotic airway epithelial cells by neighbouring epithelial cells can lead to increased inflammation. This supports the earlier observation that loss of epithelium and/or improper handling of dying epithelial cells can lead to aberrant anti-inflammatory cytokine production, thereby promoting inflammation. Indeed, knowing this in our model requires different techniques and a more detailed assessment.

Airway microcirculation undoubtedly plays a crucial role in our rat model given that rats, unlike mice, have a bronchial vasculature. Which naturally brings to attention the obvious notion that simvastatin likely had direct, protective vascular or endothelial effects, especially with respect to leukocyte transmigration into airway tissue. The statins have well-documented protective vascular effects affecting an array of biological events important in cardiovascular health and disease.

We also did not discuss airway remodelling because we used an acute 3-day smoke exposure protocol. A current manuscript in preparation documents that simvastatin prevents major hallmarks of adverse airway remodelling in rats after 4 weeks of smoke exposure. Some of the hallmarks mentioned by C. Persson such