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Early View

Perspective

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Carl Persson

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In vivo observations provide insight into roles of eosinophils and epithelial cells in asthma.

Carl Persson Laboratory Medicine University Hospital, Lund, Sweden.
Carl.persson@med.lu.se

Take home:
Exploratory in-vivo research-approaches produce unexpected discoveries and novel understanding independent of currently accepted paradigms.

Abstract.
Observations in-vivo in patients, supported by guinea-pig in-vivo data, take centre stage in this perspective. Its objective is to highlight dichotomies between asthma features observed in-vivo and accepted views involving cell/molecular biology research paradigms. For example, increased bronchial epithelial permeability is now considered a major paradigm and trait of asthma – yet, absorption of inhaled tracers has not been increased in-vivo in asthma. Such maintained barrier function in exudative asthma reflects in-vivo asymmetry of the epithelial lining as barrier between outside and inside world of molecules and cells. In desquamatory asthma, maintained epithelial tightness may be explained by in-vivo demonstrations of exceedingly patchy epithelial loss, prompt creation of plasma-derived provisional barriers, and high-speed epithelial regeneration. Acknowledged protein/peptide secretion by epithelial cells in-vitro is contrasted here with a dominant, unidirectional movement in-vivo of plasma-derived proteins/peptides (including antimicrobial peptides) to the surface of an intact epithelial lining. Further, longstanding claims that epithelium-produced adenosine is a mediator of asthma are eroded by observations in-vivo in asthmatics. Notions concerning activation/fate of mucosal tissue eosinophils illustrate additional distinctions between accepted views and in-vivo patient observations. Finally, in-vitro based paradigms preaching defect epithelial regeneration and increased permeability in pathogenesis of asthma is contrasted with experimental in-vivo observations of exaggerated epithelial regeneration, which is multi-pathogenic in its own right.
Conclusion: unexpected and challenging in-vivo observations in recent decades underpin novel insights into mucosal mechanisms in asthma.

Introduction

For about 150 years authors have championed the importance of the bronchial epithelium in asthma. Epithelial shedding and associated exudative events were among the first focal areas [1]. The potential for interactions between eosinophils and asthmatic epithelium was noted soon after the specific staining of these cells had emerged. Already 100y ago it was also speculated that epithelial-derived factors could be important in asthma [1]. The early intriguing reports dealt largely with observations of sputum and histopathology.

Whereas the epithelium and the eosinophil remain at the forefront in asthma research the revelations of late are overwhelmingly based on findings involving cultured cells and molecular biology approaches in-vitro and in-vivo. Intriguing biological data have also constituted a basis for build-up and support of currently accepted concepts on airway functions in health and asthma. We are taught about mechanisms of airway barriers, mucosal defence, eosinophil-epithelial inflammation, epithelial regeneration, and, by inference, pathogenesis of asthma. However, it appears that several paradigms have been firmly established without due consideration of observational in-vivo data. This development may only in part reflect lack of well-validated attempts at translating in-vitro data to in-vivo. It does concern, however, occasions when in-vivo observations have not agreed with acknowledged views.

Towards the end of 1990s it was widely and successfully declared that independent observation-based clinical research has nothing more to offer the medical investigator. The important revelations in that regard had already been made. However, not everyone might have agreed. In Lund we were making several unexpected in-vivo observations regarding airway microvascular, epithelial, and eosinophil aspects [2, 3], so we had to disagree[1, 4, 5]. Consistent with their unexpected nature most observations did not support the in vogue paradigms. Deeply rooted views and research paradigms were thus challenged by the novel ideas that we discussed. Our approach involved physiologically well-controlled in vivo conditions. However, likely contributing to a slow acceptance we did not provide the usual support by cell and mouse model molecular biology-data.

In this perspective the focus is foremost on observations in patients (denoted as human in-vivo data) but with support from guinea-pig in-vivo data (specified as such). Although short-term experiments in rodents may not completely reflect events in human chronic asthma, the guinea-pig models have advantages over mouse models with regard to the specific issues discussed in this review. Thus, human airway-like plasma exudation, eosinophil cytolysis, and epithelial cell loss have clearly occurred in guinea-pig- but not so in mouse models[6, 7]. In several other respects mouse models may provide attractive experimental opportunities[8].

Importantly, our continued analyses, based in no small part on an expanding source of patient in-vivo data publicised by many different authors, have underpinned our ideas. Recent years have also seen a growing acceptance of the original in-vivo findings and associated concepts. This is reflected by recent dissemination of reviews focusing on airways plasma exudation in health and disease[9] and on elimination[10, 11] and activation

of eosinophils[12, 13]. Hence, it seemed timely to discuss the commonality of dichotomies between in-vivo observations and accepted views. Several examples in the field of airway and asthma research are collated here with the objective to highlight controversies between in vivo-observations and widely acknowledged paradigms. An additional aim is to promote exploratory in-vivo research approaches. To these ends this perspective article discusses epithelial and eosinophilic aspects of the airway mucosa in-vivo in asthma. Underscoring the present theme, this perspective further includes select aspects of medical history and pharmacology of anti-asthma drugs.

Widened aspects of airway epithelium and eosinophils in asthma.

Already the physical barrier function of the airway epithelial lining is more complex than commonly discussed. For example, there is an asymmetry of the epithelial lining that needs emphasizing. It stands out by its readiness to let through macromolecules in the outward direction without altering its tightness as an absorption barrier. The mechanisms involved in this unidirectional perviousness, including supporting human patient in-vivo data, have recently been reviewed[9]. In the present perspective, particularly intriguing examples of patient observations are added to reinforce the, apparently controversial, view that an exudative disease such as asthma can have a tight barrier with a normal rate of absorption of inhaled molecules (Figure 1).

Early in-vitro studies on transepithelial migration of granulocytes reported a marked asymmetry favouring the outward basolateral-to-apical direction; further the transepithelial passage left unharmed tight junctions[14, 15]. The studies may not have focussed on elimination of granulocytes. However, such in-vitro observations agree with demonstrations of a role in-vivo of transepithelial migration (Figure 1) as a swift and silent way of ridding the airway mucosa of granulocytes in asthma[10, 11].

Less translational but widely acknowledged in-vitro observations have claimed that death of eosinophils through apoptosis, followed by phagocytosis, is the true fate of eosinophils at inflammation resolution[16]. Yet, this rapidly accepted and long-standing concept has not been confirmed in-vivo. Indeed, the proven in-vivo mode of death of airway mucosal tissue eosinophils in asthma takes another shape and is a striking mode of activation of these cells in-vivo[12, 13].

In recent years the role of epithelial lining cells as a biologically active barrier has received much attention[17]. It is clear that epithelial cells have capacity to release many molecules of interest in airway defence and disease. Some of the epithelial-derived molecules are promoted as mediators of asthma and are now targets for anti-asthma drugs. As discussed here, in-vivo efficacy of drugs is pivotal in deciding which mediator is important.

This review further underscores the possibility that plasma-derived rather than epithelial-derived molecules may be responsible for biological activities of the airway mucosal surface in-vivo. The examples concern plasma exudation-induced appearance of major defence and repair molecules including antimicrobial peptides on the surface of an intact bronchial epithelial lining in-vivo in health and asthma (Figure 1). A pronounced role also emerges at sites of epithelial denudation (largely guinea-pig in-

vivo data, see Figure 1) where exuded plasma produces a provisional barrier, which at the same time creates a milieu that promotes speedy epithelial regeneration.

Absorption of inhaled molecules is not increased in asthma patients, not even in asthma caused by epithelium-toxic chemicals – are these data enough to erode the current idea of increased epithelial permeability as a paradigm of pathogenesis of asthma?

Aspects of a maintained epithelial barrier in asthma, not included in a previous review[9], are discussed here. Toluene diisocyanate (TDI) is an epithelium-toxic chemical[18] identified as a major culprit in occupational asthma. Expectedly, inhalation of TDI produces acute effects including plasma exudation[19]. However, relatively recent studies[20, 21] demonstrate lack of increased absorption permeability in painters exposed to isocyanates, with or without occupational asthma. Painters exposed to TDI rather exhibited a tendency at reduced absorption permeability[21]. These findings significantly expand a series of in-vivo data published around 1990s indicating that asthmatic individuals in general do not exhibit increased permeability to inhaled molecules (summarized in[9]). Indeed, absorption permeability has actually been reduced[9] in studies (in asthma and in seasonal allergic rhinitis) where concomitant mucociliary clearance of tracers has been controlled. In 2018, absorption of inhaled mannitol was examined in human asthma and found to be no different from health[22]. Thus, in-vivo observations involving many cohorts and different permeability tracers have disproven the idea of increased inward permeability as a basic defect in asthma (Figure 1). Notwithstanding these patient-data, increased epithelial permeability to inhaled agents remains a widely acknowledged research paradigm and pathogenic trait of asthma[23 24].

Inflammatory stimulus-induced acute airways plasma exudation – to what extent is this associated with increased absorption-permeability of the epithelial lining?

At baseline there is a minor degree of bronchial mucosal leakage of plasma proteins. This leakage differs from the acute plasma exudation response[9] by being subject to sieving[25]. Under stable conditions small proteins may thus preferentially appear in airway surface liquids. This consideration makes large plasma proteins, notably alpha2-macroglobulin, a better index of acute plasma exudation than albumin[9]. Furthermore, especially at baseline leakage, charged entities of mucosal extracellular matrix such as glycosaminoglycans will likely bind plasma proteins[26] and thus reduce their availability for epithelial passage. Reversely, plasma proteins may bind cell-produced proteins in asthma. Thus, inducement of acute exudation of plasma has brought tissue-dwelling eosinophil cationic protein to the mucosal surface[9].

Several aspects of acute inflammatory challenge-induced plasma exudation warrant attention: the plasticity of the airway epithelial lining combined with a hydraulic mechanism evidently lets through plasma macromolecules irrespective of size and charge in one direction without altering its function as a barrier against movements of molecules in the opposite direction[9] (Figure 1). Further, the acute plasma exudation response is repeatable and reproducible. It is also short-lasting unless the challenge is aggravated[9].

Collateral evidence supports a swift and barrier-maintaining mode of bringing all plasma molecules to the intact mucosal surface: airways plasma exudation responses have not caused mucosal oedema or increased lymph transport of plasma tracers in guinea-pigs. Hence, epithelial transmission of plasma at acute responses has not created a need for drainage of the mucosal tissue of extravasated proteins[9]. The experimental in-vivo data on plasma exudation are further compatible with asthma being a disease that exhibits plasma exudation[9, 27] without increased absorption of inhaled molecules, and where evidence of actual mucosal oedema is scarce[9]. Inferentially, the ubiquitous idea that airways plasma exudation always reflects epithelial derangement and a generally increased perviousness of the mucosal barrier is not supported.

Antimicrobial peptides (AMP) and major defence and repair proteins on the surface of an intact bronchial mucosa in-vivo – are they components of plasma exudation or produced by local airway cells?

Reflecting the non-sieved nature of the acute plasma exudation response, major antimicrobial players including high and low molecular weight kininogens of the contact system[28], are exuded as components of plasma exudation in human airways [29]. Similarly, as recently reviewed in some detail[9], complement proteins, fibrinogen, fibronectin etc appear on the mucosal surface as components of a plasma exudate. To sum up, the basic shape of acute plasma exudation has the properties of a powerful defence mechanism of the intact mucosal surface. Thus, roles of plasma exudation deserve consideration in current discussions of first line mucosal defence.

In an elegant study involving atopic asthma patients, Liu and colleagues [30] demonstrated that cathelicidin, a major AMP, appeared on the bronchial surface at allergen exposure exclusively as a result of plasma exudation. Considering the ample opportunities for plasma exudation to occur at any site along the human airways it thus appears that plasma exudation could be the important mechanism that brings cathelicidin, and possibly other AMPs as well, to the surface of an intact mucosa (Figure 1). This possibility now emerges as a novel aspect in the cell culture-dominated field of AMP research.

In a study of sputum levels of several antimicrobial peptides/proteins in chronic obstructive pulmonary disease (COPD) it was only cathelicidin that was increased in association with colonisation of pathogenic bacteria at exacerbations [31]. Plasma exudation occurs in both asthma and COPD, especially at exacerbations [32]. Hence, it seems likely, as in the study by Liu et al [30], that cathelicidin was a component of exuded plasma in the COPD study[31].

Resolution of airway eosinophilic inflammation – elimination of eosinophils through apoptosis/phagocytosis that occurs in-vitro, or epithelial transmission that occurs in-vivo?

A widely acknowledged concept states that elimination of airway mucosal leukocytes occurs through apoptosis followed by phagocytosis[16]. Apoptosis of eosinophils is thus

hailed as a major mechanism of inflammation resolution in asthma. Further supporting this idea it is believed that corticosteroids and select biological drugs operate by inducing eosinophil apoptosis[16]. However, apoptotic eosinophils remain to be demonstrated in diseased airway tissues in-vivo whether steroid treatment is given or not[13, 33, 34]. Thus, the popular idea that corticosteroids operate by inducing eosinophil apoptosis is not borne out by in-vivo observations. Similarly, no compelling in-vivo data are known to support the common view that novel biologics, antagonizing IL-5, induce eosinophil apoptosis in-vivo in asthma[33, 35, 36]. There is now minimal attention to lack of in-vivo support for a role of apoptosis in the pharmacology of these major anti-asthma drug classes.

Based on sputum cell analysis eosinophil apoptosis was reported in 1996 to occur in human asthma[37]. This interesting report needs confirmation. However, a major question concerns the meaning of any leukocyte apoptosis in airway lumen material? By necessity every claimed apoptotic eosinophil in the airway lumen must have been alive as long as it dwelled in the diseased airway tissue, or it could not have migrated into the lumen. Notably, in this discussion of modes of elimination of eosinophils the lack of apoptotic eosinophils concerns the living blood-perfused airway tissue.

During the decades when the apoptosis hypothesis became established, patient in-vivo-data actually indicated that two other fates of bronchial tissue eosinophils were important[11, 13], yet they were little noticed: The silent fate of epithelial transmission and its pathogenic counterpart fate, primary cytolysis of eosinophils (cytolysis is discussed later). Large numbers of tissue eosinophils can be eliminated by trans-epithelial migration without distorting the airway epithelial barrier [38 34] (Figure 1). This mode also agrees with published, but rarely discussed observations at resolution of inflammation in-vivo in asthma[10 11]. Interpretation of granulocyte numbers in the air space would depend on the phase of disease processes, whether inflammation is developing, persistent or resolving.

The epithelial transmission of eosinophils can occur randomly between epithelial cells[38 34]. This aspect would be important both for inflammation resolution and for the ability of these cells to be active in first line antimicrobial defence at any site of mucosal surface attack. The defence aspect has been particularly well demonstrated with regard to neutrophils emerging on the epithelial surface of urinary bladders[39]. In the gut mucosa the interest in epithelial crossing of neutrophils has an additional focus on anchorage of the leukocytes to the apical epithelial membrane in defence and inflammation[40]. Hulbert et al[41], who underscored roles in first line mucosal defence, claimed that neutrophils were transmitted across the airway epithelial lining in a train fashion at certain sites. - Studies seem warranted to better define roles in-vivo of epithelial transmission of granulocytes both for defence operations at the surface of an intact airway mucosa and for silent elimination of these cells at inflammation resolution.

Cytolysis of eosinophils and occurrence of clusters of free eosinophil granules (C-Fegs) – is this an artefact or a major mode of eosinophil activation in-vivo?

Occurrence and potential importance of eosinophil cytolysis tally with the present theme of original in-vivo discoveries. Whereas apoptotic eosinophils could qualify for a

phantom phenomenon, another shape of eosinophil death, primary cytolysis, abounds in-vivo in diseased tissues[13, 42 43]. Many tissue eosinophils in asthma are thus eliminated by primary cytolysis (without involvement of apoptosis). Importantly, this mode of death entails ultimate activation and potential pathogenic effects of these cells in diseased airways, including an intriguing association with epithelial shedding[13, 43] (Figure 1).

Prior to and in parallel with the decades of hegemony of the apoptosis mechanism, the cytolysis mode of eosinophil death was considered an artefact or, perhaps most commonly, not considered at all[44]. A turning point conquering the disbelief was the understanding and the demonstration beyond doubt that the cytolysis was an inducible, non-artefact in-vivo phenomenon[44]. As such it was readily identified in-vivo by occurrence of clusters of free eosinophil granules (C-Fegs) in the airway mucosa[13, 45] (Figure 1). The ultrastructural features of cytolysis include chromatolysis and cell membrane rupture, allowing the spilling of cell nucleus and cytoplasm contents. Most conspicuous are the C-Fegs, containing and releasing potent proteins[44].

C-Fegs are intriguingly associated with childhood and adult asthma including apparent associations with epithelial sloughing and severity of the disease[13 43]. A role of eosinophils in epithelial derangement and shedding has further been suggested based on epithelium-toxic actions of granule proteins[46, 47] known to be released from C-Fegs in-vivo in human diseased airways[13]. Weller and colleagues have significantly expanded the research area by demonstrating that C-Fegs can be separately regulated as release-competent organelles[48].

Occurrence and potential importance of primary cytolysis of eosinophils are increasingly being accepted[12, 13, 43]. One piece in this development is the recent report by leading champions of the eosinophil apoptosis paradigm acknowledging that apoptotic eosinophils may rarely occur in human diseased airway tissue[49, 50]. There have also been publications reporting that molecular mechanisms of eosinophil apoptosis may actually have dealt with primary cytolysis of eosinophils[33].

The current adoption of the paradigm of eosinophil cytolysis by experts on the biology of eosinophil apoptosis[51] is both telling and welcome. A controversy may remain regarding the role of primary cytolysis in formation of eosinophil extracellular nets[49, 52]. However, the current degree of acceptance of eosinophil cytolysis as a major mechanism should advance our knowledge of the molecular biology of eosinophil cytolysis. Perhaps time is growing ripe to complement the mere counting of blood eosinophils[53] with a test of their propensity to undergo primary cytolysis? The aim would be to move towards precision in identifying patients whose disease is eosinophil-driven.

Highly active plasma-derived barriers over a denuded, but intact, basement membrane cater for safe and speedy epithelial regeneration in-vivo - may exaggerated epithelial regeneration, rather than the current paradigm of defect repair, characterize asthma?

Lemarchand et al[54] reported that acute asthma differed from stable asthma by exhibiting a transient increase in bronchial clearance of inhaled tracer molecules. Hence, in the

acute condition, where epithelial shedding may be much increased[55], bronchial permeability to inhaled molecules may be increased. This possibility needs confirmation. However, the transient nature of the permeability increase together with the lack of increased absorption in stable asthma (discussed above) would argue against the currently established notion of defect epithelial repair in asthma[56, 57]. Accordingly, it was recently suggested that asthma may be a disease where exaggerated epithelial regeneration activities operate to oppose escalating epithelial barrier breaks once epithelial denudation has occurred[9].

Asthma-like denudation in-vivo in guinea-pigs is non-sanguineous and involves no injury to the basement membrane[58]. At such denudation sites epithelial regeneration starts promptly[58]. Studies of epithelial shedding-repair in vivo, where the basement membrane has been injured, have recorded a considerably delayed start of regeneration and a relatively slow reestablishment of a new cell cover[59, 60]. This is so even if commendable attempts have been made to produce tiny areas of denudation[60].

Lack of bleeding does not mean lack of wound protection. Guinea-pig in-vivo studies demonstrate that promptly following non-sanguineous removal of epithelium from the intact basement membrane, plasma proteins exude and produce a highly active barrier kept together at the denudation site by a plasma-derived fibrin-fibronectin net. This provisional barrier is continuously supplied by bulk plasma and leukocytes, including activated neutrophils [58, 61]. A milieu of exuded plasma proteins may be a prerequisite for speedy repair. Further, a provisional, plasma-derived barrier at patchy sites of shedding, together with high-speed epithelial restitution, likely contributes to a maintained absorption barrier in asthma (Figure 1).

Following asthma-like denudation in guinea-pig airways in-vivo, all types of epithelial cells bordering the spot of exposed basement membrane, notably including columnar ciliated and secretory cells, promptly dedifferentiated into poorly differentiated, flattened, mesenchymal- and basal cell-like cells[58]. By a joint effort of all neighbouring cells, the restitution of a cell cover would thus be optimized in-vivo (Figure 1). This observation was novel at the time. The regeneration cells moved in a tethered fashion and at a high speed in a plasma exudate milieu[58]. By 15 min the front of migrating regeneration cells had moved 30-50um. Basal cells have commonly been considered stem cells responsible for epithelial regeneration in the lung[62]. By advanced techniques it has now been confirmed that also other local epithelial cell types than basal cells have capacities to participate in airway epithelial regeneration[63]. Apparently, this recent development has not needed the support provided by the original in-vivo observations.

Micrographs illustrating extensive denudation may reflect areas with numerous tiny denudation spots – is patchy epithelial sloughing key to maintained mucosal tightness in-vivo in asthma?

Increased sloughing of epithelium in asthma is evident from analyses of sputum samples. Epithelial derangement and denudation have also been illustrated in micrographs of childhood and adult asthma [64, 65]. A current discussion concerns the possibility that denudation graphs may represent epithelial fragility just as much as actual denudation[66]. Indeed, it may well be both. Experimental airway challenges in

guinea-pigs and dogs with allergen and dry air have produced many tiny sites of denudation[42, 67, 68], most clearly demonstrated in helicopter views of tissue whole-mounts[42]. Sectioning of airways with many small spots of denudation has produced much increased areas of complete denudation[42] suggesting the novel possibility that common denudation micrographs of asthmatic bronchi could represent areas scattered with patchy denudation spots.

Patchiness of epithelial loss in asthma is an infrequently discussed phenomenon. However, its occurrence is supported by in-vivo observations of epithelial cells clumped together in sputum. This feature was repeatedly observed at the turn of the 19th century[1]. It was studied in greater detail in the 1960s. Honouring the name of one of his patients Naylor[55] named clumps of epithelial cells occurring in sputum from asthma patients Creola bodies (Figure 1). Creola bodies are commonly defined by their content of the easily recognisable ciliated cells. However, also other types of epithelial cells may well be present. Although selective loss of ciliated cells may occur in asthma[69] small clusters of both columnar and basal epithelial cells may leave the basement membrane together[46] to produce Creola bodies.

Pathogenesis of asthma - exaggerated epithelial regeneration rather than increased permeability?

Epithelial shedding seems particularly pronounced at exacerbations[55, 70]. Naylor thus observed Creola bodies in nearly all asthmatics that delivered sputum during asthma exacerbations or the week after an asthma attack. The number of Creola bodies in only two smears could be well over 100[55]. Hence, numerous sites of shedding were likely involved suggesting that also in acute and severe asthma there is an exceedingly patchy mode of epithelial denudation (Figure 1). Supporting the association between epithelial loss and disease severity, patients with increased numbers of Creola bodies tended to have a longer stay in hospital[55]. In agreement, Ogata et al[70] demonstrated that a sputum Creola body score correlated with both sputum levels of ECP and the number of hospitalized days needed for remission after asthma attacks.

Yamada and colleagues have made interesting observations on occurrence of Creola bodies in sputum samples obtained from infants[71, 72]. One study focused on 23 hospitalized, wheezing infants with a mean age of about 5 months. They were divided into two groups depending on presence or absence of Creola bodies. In the first month following discharge asthma symptoms were much higher in the group positive for Creola bodies. Twelve (80%) of these children, but none in the group that lacked Creola bodies, were diagnosed with infantile asthma during a 2-year follow-up period[71].

In a second study Yamada et al [72] examined occurrence of Creola bodies in infants admitted with acute respiratory syncytial virus (RSV) infection. Potential roles of infection with RSV in inception of asthma have received much attention but data in this field have not been consistent[73]. In their study of RSV-infected infants, Yamada et al[72] demonstrated that only those who presented with Creola bodies in aspirated sputum exhibited a significant risk (relative risk of 3 and 7, respectively) of developing recurrent wheezing and asthma during follow-up at 2 and 5 years. Hence, the authors

strengthened their idea of a prognostic value of epithelial shedding in predicting future development of asthma.

Lethal RSV infections of infants have been characterized by pronounced shedding of epithelial cells. Sloughed epithelium together with admixed fibrin and some mucus have occluded the bronchial lumen in these cases[74]. Of note, the epithelial sloughing in cross-sections was patchy[74]. This finding further tallies with demonstrations of quite patchy occurrence of infections in bronchial epithelial cells exposed to rhinovirus[75].

As reviewed elsewhere, the mere removal of epithelial cells from a small area of intact basement membrane in-vivo in guinea-pig airways is followed by several events of interest in relation to features of asthma[9, 76, 77]. Reflecting common regeneration stages epithelial cell remodelling occurs at regeneration sites[58]. Importantly, additional effects of inflammatory as well as remodelling nature are induced: (A) Plasma is exuded. (B) Mucus is secreted. (C) Neutrophils are recruited and activated. (D) Eosinophils already present in local airway tissues undergo primary cytolysis. (E) Proliferation of subepithelial fibrocytes/smooth muscle is induced. (F) By repeated epithelial denudation-regeneration sequences the reticular basement membrane is thickened[43, 76, 78]. Molecular pathways involved in the regeneration-induced effects listed above have not been determined. Whereas such a limitation may prevent establishment of a strong research paradigm it does not invalidate the in-vivo based idea of costly epithelial regeneration in asthma as a pathogenic factor in its own right.

Anti-asthma drugs and proposed mediators of asthma emanating from stressed epithelium – is the claimed role of adenosine eroded by in-vivo observations in asthma?

Since their arrival, the modus operandi of antiasthma drug classes has naturally been explained in the light of contemporary ideas of disease features. When asthma was considered a disease of reduced nerve activity, strong coffee (containing methylxanthines) was active because it was a stimulant[79]. When asthma was thought to reflect 'vasomotor ataxia', adrenalin acted because it was a vasoconstrictor[1] etc. Now drug- and disease mechanisms are being linked based on cell and molecular biology observations. However, the discussion above on roles of eosinophil apoptosis suggests that not all currently accepted views on pharmacological mechanisms might stand the test of critical in-vivo evaluations.

Several autacoids released from stressed and injured airway epithelium have attracted pharmacological interest. Thymic stromal lymphopoietin (TSLP) is known to be induced by human epithelial cells at rhinoviral infection[80, 81] and injury-repair[82 83]. Since clinical efficacy of a biologic anti-TSLP drug in exacerbating asthma was recently demonstrated[84], TSLP now stands out as a drug target. Adenosine is also emanating from injured epithelium[85] and has for a long time been viewed as an important mediator of asthma[86]. The idea is claimed to be supported by cell and molecular biology data (of unknown clinical relevance) and, more concretely, by propositions that theophylline, a universal adenosine antagonist, is active in asthma through its antagonism of adenosine[86]. This dogma was not helped by the "surprise" arrival of enprofylline, a

xanthine chemically very close to theophylline but dramatically different regarding adenosine antagonism and extrapulmonary actions[87].

Enprofylline's distinct profile in humans in-vivo reflected its lack of inhibition of adenosine-induced effects[87]. Regarding anti-asthma efficacy, ranging from inhibition of allergen challenge-induced late phase reaction to improvement over placebo at maintenance treatment, the two xanthines were both clinically efficacious and to the same extent[88-90]. The only difference in asthma was detected when theophylline, but not enprofylline (sic), preferentially inhibited adenosine challenge-induced bronchoconstriction in patients[91] . Unfortunately, in seminal discussions these data have been turned around and referred to as "preferential inhibitory effect of intravenous enprofylline on adenosine-induced bronchoconstriction"[86]. The crucial fact is the opposite.

Reflecting its efficacy combined with lack of adenosine antagonism, enprofylline reduced bronchoconstriction produced by histamine and adenosine challenges equally. However, theophylline, being a potent adenosine antagonist, was the drug that preferentially inhibited the adenosine-induced effect[91]. Hence, the two xanthines, at relevant plasma concentrations, were successfully used as tools to critically assess involvement of adenosine in asthma. As agreed in the original publication[91], the data together with the clinical efficacy of enprofylline[88-90] seriously eroded the idea that adenosine was an important mediator of asthma. In summary, clinical effects together with pharmacological features of anti-asthma drugs demonstrate that TSLP is an important epithelium-derived player in asthma whereas a role of adenosine is not supported. Increased expression of TSLP in repairing epithelium[83] is further consistent with a pathogenic role of exaggerated epithelial regeneration in asthma.

Select early contributions to our understanding of asthma and its treatment may not have received due attention – tribute to Salter and Solis-Cohen.

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I think we can still learn from Henry Hyde Salter (1827-1871), an astute original observer of numerous important features of asthma. Without the need of molecular explanations his original descriptions of "Asthma, its pathology and treatment"(cited in[79 92]) have lasting value. It is no coincidence that he could contribute so much. Expressing that colleagues "had done more in the way of reading each other's books than scrutinizing their own patients" Salter strongly advocated "simple reading of nature". Thus, he was clear in his criticism of the common occurrence of "unquestioning inheritance and adoption of received notions". His teachings also involved encouraging advice: "respect for authority, however high should never be pushed to concessions of anything positively observed". However, several of Salter's astute accounts may not have been widely noticed. This concerns his observations relating to major anti-asthma drug principles[79]. Also, his detailed observations of asthma exacerbations evoked by common cold may have been missed and confused with his important notes on the influence of cold air [92]. For nearly a century Solomon Solis-Cohen, who actually gave an intriguing description of glucocorticosteroid-like therapeutic features of oral intake of "adrenal substance" in asthma, was incorrectly considered to have made a first demonstration of effects of adrenaline [1]. These examples of medical history aspects may be significant by revealing how easy it is to miss essential in-vivo observations. As discussed above, there

is a risk that also some of the currently held notions and associated research paradigms are similarly unsupported by well-established data in-vivo.

How can research paradigms thrive in the face of contradictory in-vivo observations?

Developing fast since the final decades of 1900s biotechnical advances at molecular and cellular levels have created an explosive development of biological research opportunities. Naturally, research approaches have been selected where the novel techniques could be well applied. Innovative cell culture experiments often combined with versatile mouse models thus came to dominate the airway research scene from which an avalanche of new data emerged. The possibility that actual disease mechanisms might not be reflected in the available test systems received less attention. Hence, a risk of imbalance in medical research became evident[5].

New biological information was not only intriguing; thanks to increasingly available molecular schemes, data could also be communicated with proposed chains of events whereby biology was transformed into apparent medical importance. Complementary demonstration of individual molecules in patient airways might then suffice to produce high impact definitive reports. The language advantage inherent in the reductive approaches cannot be underestimated. – Unsupportive observational in-vivo data in patients may have been available. However, lacking in molecular explanations they carried less weight. Language disadvantage may in part explain why in-vivo observations would not be considered convincing enough to create, or refute, paradigms.

The new biology also received hegemony in part based on overstatements inferring that what could be learnt by observation-based in-vivo events had already been well established. Embarking on the increasingly available, novel opportunities with purported medical importance became exceedingly rewarding. Under such circumstances unbiased and de novo considerations of what has been observed in-vivo in patients might not receive the highest priority. It seemed acceptable to be history-less regarding disease-relevant observations in-vivo.

Communication of biological data has involved explicit promises. Thus, suggested paths towards treatment opportunities have flourished in the literature. However, those who take proposed novel drug targets seriously would be wise to carefully validate the reported data. It was recently noted that more than 60% of drug targets reported in high impact journals were not even reproducible[93]. Such flaws may tell about the attraction of discoveries at the molecular level for researchers and journals alike.

Importantly, limitations of popular concepts such as those discussed in this article shall not detract from the fact that molecular biology and cell studies have been and will be successful in creating novel medically important concepts, also leading to significant treatment opportunities. One such advance, perhaps even exceeding expectations of experts in the field[81], is the recent demonstration that a biological drug, specifically antagonizing epithelial-derived TSLP, inhibits exacerbations of asthma[84].

Conclusion

This perspective discusses a variety of epithelial and eosinophil features of the airways in asthma. The focus is on in-vivo observations that underpin ideas that deviate from, or complement current notions and research paradigms. Inferentially, this review highlights the potential importance of unexpected and challenging in-vivo discoveries. The specific issues reflect a biased selection of discussion points. However, the fact that an outlook largely limited to personal encounters has identified these instances of dichotomies between in-vivo observations and commonly accepted paradigms suggests that the phenomenon has a degree of general applicability.

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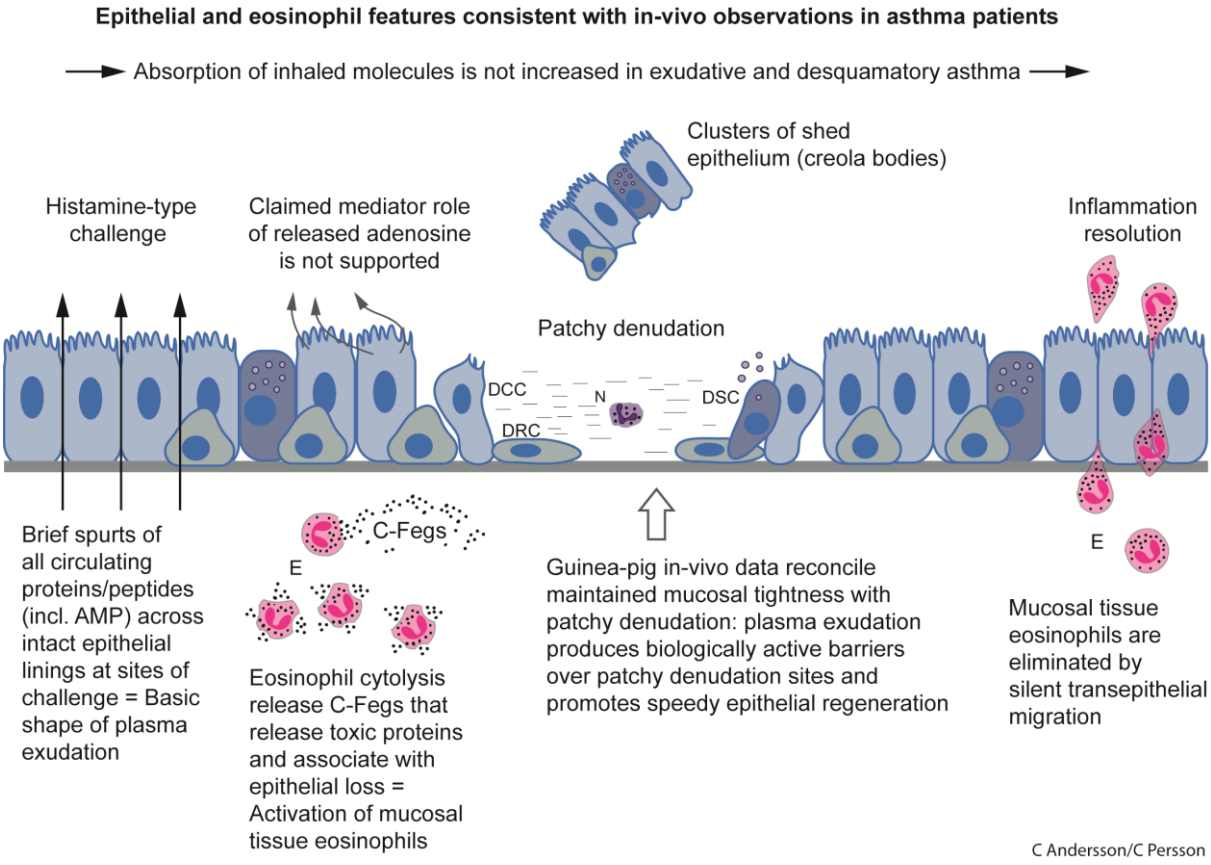

Legend figure 1.

Epithelial and eosinophil features demonstrated by and consistent with in-vivo observations in asthma patients are depicted. Features of the epithelial lining such as lack of increased absorption, exudative response to histamine-type challenge, patchiness of epithelial loss, events following patchy desquamation, and involvement in inflammation resolution are indicated above the epithelium. Features involving also the superficial microcirculation and mucosal eosinophils, respectively, are indicated below the epithelium. The illustrated epithelial regeneration events at denudation patches represent observations that so far have been made in guinea-pig airways in-vivo. Other features in this figure represent in-vivo observations made in human asthma as well as in guinea-pigs. As discussed in this perspective article, there are distinct dichotomies between roles of the depicted in-vivo events and established paradigms that are largely built on observations in-vitro.

Abbreviations:

AMP, antimicrobial peptides; C-Fegs, clusters of free eosinophil granules; DCC, dedifferentiating ciliated cell; DRC, dedifferentiated regeneration cells; DSC, dedifferentiating secretory cell; N, neutrophils; E, eosinophils.