Key role of dysregulated airway epithelium in response to respiratory viral infections in asthma

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To the Editor:

We congratulate Ravi *et al*. [1] for their recent paper in *ERJ Open Research*, "Imprinting of bronchial epithelial cells upon *in vivo* rhinovirus infection in people with asthma". We would like to discuss their study and make some contributions.

In this article, bronchial epithelial cells (BECs) obtained from asthma patients and healthy individuals before and after *in vivo* rhinovirus (RV)-A16 exposure were cultured in submerged conditions. BECs were stimulated with tumour necrosis factor and interleukin (IL)-17A to stimulate the production of inflammatory cytokines including CXCL-8, IL-6, granulocyte colony-stimulating factor and CXCL-10, which were analysed, leading to the description of an epithelial hyperresponsive phenotype. This epithelial hyperresponsiveness increased in BECs of asthma patients after RV-A16 exposure, even in those who showed no epithelial hyperresponsivity prior to RV-A16 challenge. This did not occur in healthy individuals. The authors concluded that epithelial hyperresponsiveness is an intrinsic defect in the bronchial epithelium of asthma patients, which increases upon rhinovirus exposure, and that this is not present in healthy individuals [1].

Previously, Wark *et al.* [2] also used an *in vitro* model of RV-16 infection of BECs, cultured in submerged conditions, to show that cells from asthma patients produce lower levels of interferon-β that was associated with increased virus replication and cell lysis, compared to those from healthy donors. More recently, *ex vivo* RV-A1 infection of asthma BECs, cultured at the air–liquid interface (BECs-ALI), showed delayed innate antiviral immune responses compared to those from healthy donors [3].

Epithelial integrity loss, basement membrane thickening, and goblet cell and submucosal gland enlargement contribute to airway remodelling [4]. Airway remodelling is related to physiological airway dysfunction [5] and may predispose asthma patients to exacerbations [6] upon exposure to respiratory viral infections. Interestingly, Kuo *et al*. [7] demonstrated that *in vitro* RV-16 infection of submerged BECs from healthy donors stimulated the production of extracellular matrix (ECM) proteins, including perlecan and collagen V, and matrix-bound vascular endothelial growth factor, mediated through Toll-like receptors. Increased ECM protein deposition is a major characteristic of airway remodelling in asthma. Hence, RV infection may facilitate airway remodelling that may be more distinct in asthma.

We have also observed intrinsic changes in the epithelium of asthma donors, and it is of interest that some differences remain after primary cell culture and passage [8]. Researchers have consistently shown dysregulated epithelial homeostasis, including a greater proportion of basal and fewer ciliated cells, which are associated with deficient innate immune responses, in people with asthma [2, 9, 10].

We also believe that the epithelium plays a key role in asthma exacerbations induced by respiratory viral infections. Since neither genetics nor environmental factors fully define the aetiology of asthma, we have focused on examining epigenetic regulatory mechanisms.

Epigenetic factors, including DNA methylation, histone modifications and noncoding RNA, determine heritable regulation of gene transcription that does not require alterations in gene sequence [11]. Pech *et al.* [12] showed that *in vitro* RV infection of nasal epithelial cells in submerged culture, resulted in differential

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DNA methylation and subsequently, differential mRNA expression in children with asthma. Two asthma-specific genes affected were human leukocyte antigen B-associated transcript 3 (*BAT3*) and neuraminidase 1 (*NEU1*), which are involved in host immune responses against RV infection and asthma pathogenesis.

We have also recently demonstrated that expression of microRNA-22 (miR-22) differs in BECs-ALI of asthma patients after *ex vivo* influenza A virus (IAV) H1N1 stimulation (multiplicity of infection 5) compared with non-asthma donors [8]. In BECs-ALI from non-asthma donors 24 h post-infection, miR-22 expression increased significantly, associated with the suppression of CD147 and histone deacetylase (HDAC)4 mRNA and protein levels, and downstream matrix metalloproteinase (MMP)-9 mRNA and protein levels. In contrast, in cells from asthma patients, miR-22 remained unchanged while CD147 expression increased, and HDAC4 and MMP-9 remained unaffected. IAV H1N1 induced increases in *SP1* and *c-Myc* transcription factors that may underpin the induction of CD147 in asthma donors [8]. CD147 (EMMPRIN/basigin/HAb18G) induces the production of MMPs, such as MMP-9, with important roles in airway remodelling and inflammation [13]. Epithelial cells are the major source of CD147 [13]. We determined that ectopic overexpression of miR-22 in BECs of asthma patients led to the suppression of CD147 expression, *ex vivo* [8], indicating the therapeutic potential of miR-22 mimics as a treatment of influenza-induced asthma exacerbations.

In our model, BECs at passage 2 were differentiated over 23–25 days and during this period, cell media was refreshed frequently [8]. We feel that this indicates that differential expression of miR-22 and its targets in epithelial cells of asthma patients after IAV infection may be intrinsic. Further, IAV H1N1 infection showed no effect on miR-22 expression in epithelial cells in submerged culture conditions, from asthma and non-asthma donors, indicating that this effect was associated with differentiated cells. The different profile of miR-22 expression in differentiated epithelial cells from non-asthma patients may therefore indicate a self-defence mechanism against aberrant epithelial responses through suppressing CD147 and HDAC4, which is compromised in epithelial cells of asthma patients [8].

Overall, our work thus supports the findings of Ravi *et al*. [1] which show intrinsic epithelial dysregulation in asthma patients. Structural and functional abnormalities maintained during weeks of culturing may be valuable in defining the potential for new therapeutic approaches in asthma and yield insights into the susceptibility of people with asthma to viral-induced exacerbations.

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