



Inhibition of Pim1 kinase reduces viral replication in primary bronchial epithelial cells

To the Editor:

Respiratory viral infections are responsible for 85% of asthma exacerbations, which in turn lead to increased morbidity and mortality, causing a high societal and economic burden [1, 2]. Asthma patients have been found to be more susceptible to viral infections compared with non-asthmatic individuals [3]. It has been postulated that a reduced induction of apoptosis in virally infected airway epithelial cells from asthmatic patients might play a role in this increased susceptibility to viral infections [4]. Building on the role of apoptosis in respiratory viral infections, we tested whether the inhibition of a well-known survival pathway active in airway epithelial cells could reduce viral replication. To this end, we used an *in vitro* approach and infected primary bronchial epithelial cells (PBECS) from healthy volunteers with human rhinovirus (HRV)-16 in the absence or presence of a highly specific pharmacological inhibitor for Pim1 kinase [5]. Pim1 kinase is a constitutively active serine/threonine kinase known to be involved in cell survival by increasing the threshold for apoptosis [6, 7]. We have shown previously that Pim1 kinase is highly expressed in the bronchial airway epithelium and that pharmacological inhibition of Pim1 kinase increases the sensitivity of bronchial epithelial cells to cell death upon challenge with cigarette smoke extract [8]. Therefore, we hypothesised that inhibition of Pim1 kinase activity in virally infected PBECS would enhance the onset of cell death, resulting in reduced viral replication.

Infection of monolayer cultures of PBECS with HRV-16 at a multiplicity of infection of 1 resulted in highly increased viral copy numbers 8 h and 24 h after infection compared with mock-infected controls (fig. 1a and b, respectively). Except for some low background signal, no viral RNA was detectable in either sham or ultraviolet light irradiated HRV-16 infected control PBECS or in PBECS exposed to the inhibitor in the absence of HRV-16. Interestingly, inhibition of Pim1 kinase activity significantly reduced viral RNA copy numbers, leading to a reduction in viral RNA load of ~60–65% at both time points. The reduced viral replication upon inhibition of Pim1 kinase activity was further supported by a marked suppression of the amount of viral particles released by the PBECS 24 h after viral infection (fig. 1c). Viral particles were only observed in PBEC cultures infected with HRV-16. Inhibition of Pim1 kinase activity resulted in an almost 10-fold reduction in the amount of viral particles released from the PBECS. A comparable effect was observed with the ATP-binding-mimicking Pim1 kinase inhibitor SMI-4a, suggesting that the reduction in viral replication is Pim1 kinase specific (data not shown) [9]. Next, we aimed to test whether the observed reduced viral replication upon inhibition of Pim1 kinase activity was a consequence of an increased anti-viral response or enhanced induction of apoptosis. To this end, we first examined the anti-viral response and assessed the mRNA expression levels of the innate anti-viral cytokines interferon (IFN)- β and interleukin (IL)-29, 8 h after infection. Although expression levels were relatively low in the PBEC monolayer cultures, in agreement with KHAITOV *et al.* [10], inhibition of Pim1 kinase activity resulted in increased mRNA expression of IFN- β (fig. 1d) and IL-29 (fig. 1e) compared to the non-treated HRV-16 infected control cultures. Evaluating the activation of the anti-viral inflammatory response by analysing the mRNA expression of the IFN-inducible genes IFN γ -induced protein (IP)-10 and RANTES, we observed a significantly decreased expression of both IP-10 (fig. 1f) and RANTES (fig. 1g) in HRV-16 infected PBEC cultures treated with the Pim1 kinase inhibitor compared to control cultures. As shown in figure 1h and i, IP-10 and RANTES protein levels in supernatant were extremely low and we were not able to detect IP-10 or RANTES in all samples, which might be a consequence of the relatively short half-life, as determined for RANTES [11]. While we could not perform statistical evaluation of these data, IP-10 (fig. 1h) and RANTES (fig. 1i) protein levels seem to follow mRNA expression levels, with increased protein levels upon infection with HRV-16 and a subsequent decrease in protein levels upon concurrent inhibition of Pim1 kinase activity. Since the increased innate anti-viral response (mRNA expression of type I and III IFNs) observed upon inhibition of Pim1 kinase in virally infected PBECS did not correspond with an increased activation of the anti-viral inflammatory response, we next examined the role of cell death in reduced viral replication upon inhibition of Pim1 kinase activity. Based on the reduced viral RNA replication in the presence of the Pim1 kinase inhibitor observed at 8 h post-infection, we sought to

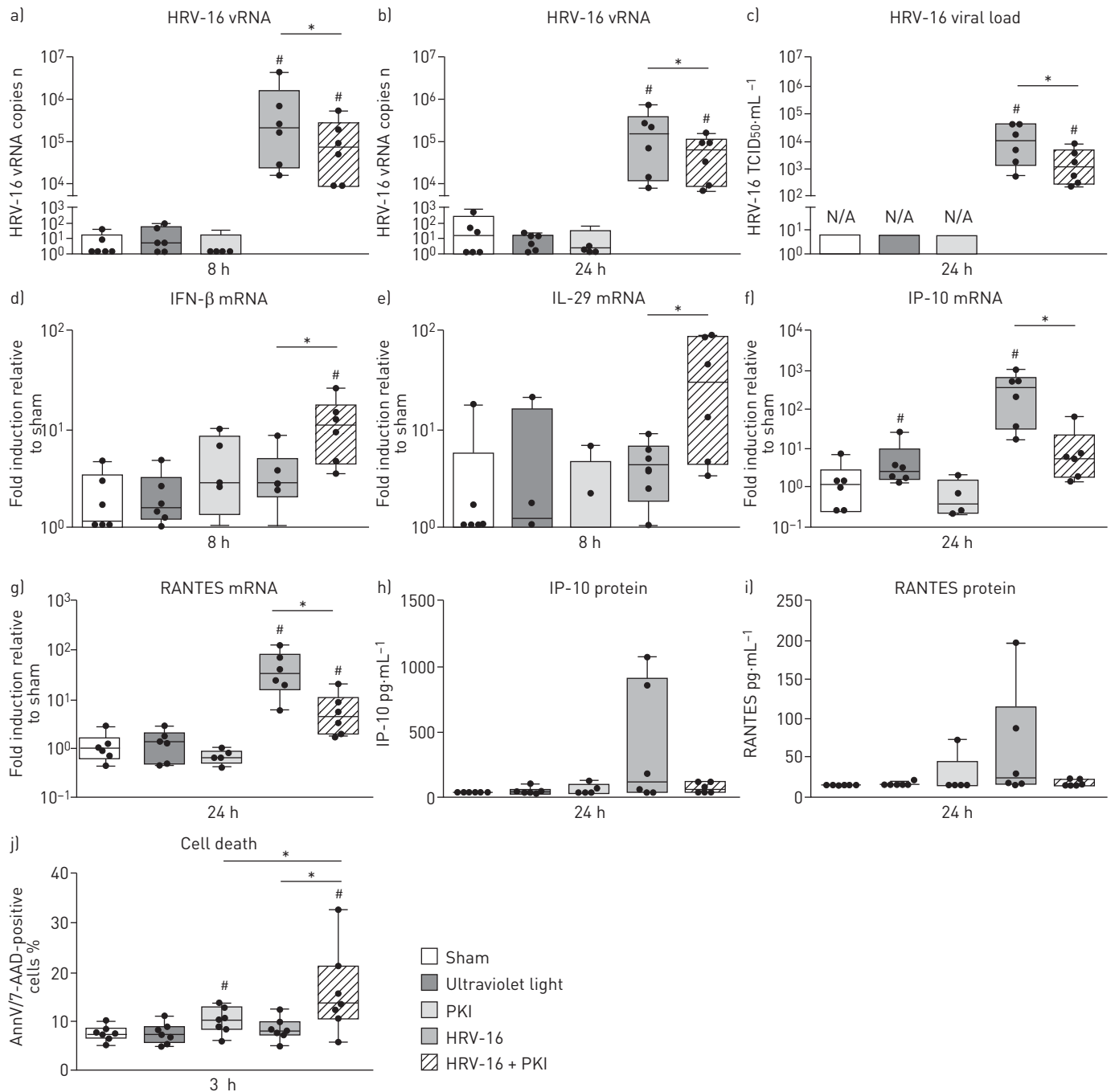


FIGURE 1 Infection of monolayer cultures of primary bronchial epithelial cells with human rhinovirus (HRV)-16 in the presence of a specific pharmacological Pim1 kinase inhibitor (PKI) reduces viral replication by augmenting the onset of cell death. Viral (v)RNA copy numbers were determined using reverse transcriptase (RT)-quantitative (q)PCR a) 8 h and b) 24 h after infection. c) Release of viral particles was measured by 50% tissue culture infective dose (TCID₅₀) 24 h after infection. mRNA expression of the innate anti-viral genes d) interferon (IFN)-β and e) interleukin (IL)-29 8 h after infection was assessed using RT-qPCR. mRNA expression of the IFNγ-induced genes f) IFNγ-induced protein (IP)-10 and g) RANTES 24 h after infection was measured using RT-qPCR. Protein levels of h) IP-10 and i) RANTES 24 h after infection were analysed using commercially available ELISA (R&D Systems, Abingdon, UK). j) The amount of cell death 3 h after infection was assessed by analysing annexin V (AnnV) and 7-amino-actinomycin D (AAD)-positive cells using flowcytometry. The experiments were repeated six or seven times. Data are presented as median and range. Statistical significance was tested using the paired two-sided Wilcoxon matched pairs signed rank test. N/A: not applicable. *: p<0.05; #: p<0.05 compared to sham-infected controls.

analyse the induction of cell death at earlier time points. This was determined experimentally and shown to be optimal 3 h after infection. Therefore, we determined the induction of cell death upon infection with HRV-16 by analysing the presence of phosphatidylserine on the outer cell membrane and loss of cell membrane integrity 3 h after infection. As shown in figure 1j, no significant differences could be observed in cell death when cells were infected with HRV-16 compared with controls. However, cells infected with

HRV-16 in the presence of the Pim1 inhibitor showed a significant increase in the amount of cell death compared with cells infected with HRV-16 alone or treated with the Pim1 inhibitor in the absence of HRV-16 (fig. 1j), suggesting that Pim1 inhibition sensitises the PBECs to apoptosis induction upon HRV-16 infection.

This study shows for the first time that inhibition of Pim1 kinase activity reduces viral replication and release of viral particles in cultured PBECs from healthy volunteers by enhancing the induction of cell death upon viral infection. The observed role of cell death in viral infections is in line with previous studies showing that the early apoptotic response is an important feature of the anti-viral response, limiting viral replication and subsequent viral spread [4, 12]. While decreased cell survival alone could explain the reduction in viral replication, inhibition of Pim1 kinase also resulted in a slight increase in mRNA expression of IFN- β and IL-29, 8 h after infection with HRV-16. However, mRNA expression levels of IFN- β and IL-29 in virally infected PBEC cultures treated with the Pim1 kinase inhibitor are very low and were not sufficient to enhance levels of the IFN-inducible genes IP-10 and RANTES, 24 h after infection. The levels of IP-10 and RANTES probably follow the amount of virus present in the cells, which is markedly reduced upon inhibition of Pim1 kinase activity (fig. 1b, c) [13]. Consequently, we feel that the inhibition of viral replication by inhibition of Pim1 kinase is largely achieved through its effect on reduced cell survival of HRV-16 infected cells. Taken together, our findings imply that inhibition of Pim1 kinase inhibits viral replication and viral particle release from HRV-infected PBECs. Therefore, Pim1 inhibition could be a new therapeutic approach in the treatment of viral-induced asthma exacerbations. Further work is now required to assess the effect of Pim1 kinase inhibition in PBECs from patients with severe asthma and to explore the underlying mechanisms of suppression of viral replication and the role of an enhanced innate anti-viral response upon inhibition of Pim1 kinase activity. Also of interest is testing the broader potential of inhibition of Pim1 kinase activity to affect the replication of other respiratory viruses, such as influenza and respiratory syncytial virus, also commonly detected in virally induced asthma exacerbations [1].



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Inhibition of Pim1 kinase activity reduces viral replication by inducing the onset of bronchial epithelial cell death <http://ow.ly/IJWGD>

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References

- 1 Jackson DJ, Sykes A, Mallia P, *et al.* Asthma exacerbations: origin, effect, and prevention. *J Allergy Clin Immunol* 2011; 128: 1165–1174.
- 2 O'Byrne PM. Therapeutic strategies to reduce asthma exacerbations. *J Allergy Clin Immunol* 2011; 128: 257–263.
- 3 Cakebread JA, Xu Y, Grainge C, *et al.* Exogenous IFN- β has antiviral and anti-inflammatory properties in primary bronchial epithelial cells from asthmatic subjects exposed to rhinovirus. *J Allergy Clin Immunol* 2011; 127: 1148–1154.
- 4 Wark PA, Johnston SL, Bucchieri F, *et al.* Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005; 201: 937–947.
- 5 Pogacic V, Bullock AN, Fedorov O, *et al.* Structural analysis identifies imidazo[1,2-b]pyridazines as PIM kinase inhibitors with *in vitro* antileukemic activity. *Cancer Res* 2007; 67: 6916–6924.

- 6 Macdonald A, Campbell DG, Toth R, *et al.* Pim kinases phosphorylate multiple sites on Bad and promote 14-3-3 binding and dissociation from Bcl-XL. *BMC Cell Biol* 2006; 7: 1.
- 7 Nawijn MC, Alendar A, Berns A. For better or for worse: the role of Pim oncogenes in tumorigenesis. *Nat Rev Cancer* 2011; 11: 23–34.
- 8 de Vries M, Heijink IH, Gras R, *et al.* Pim1 kinase protects airway epithelial cells from cigarette smoke-induced damage and airway inflammation. *Am J Physiol Lung Cell Mol Physiol* 2014; 307: L240–L251.
- 9 Lin YW, Beharry ZM, Hill EG, *et al.* A small molecule inhibitor of Pim protein kinases blocks the growth of precursor T-cell lymphoblastic leukemia/lymphoma. *Blood* 2010; 115: 824–833.
- 10 Khaitov MR, Laza-Stanca V, Edwards MR, *et al.* Respiratory virus induction of alpha-, beta- and lambda-interferons in bronchial epithelial cells and peripheral blood mononuclear cells. *Allergy* 2009; 64: 375–386.
- 11 Hillyer P, Male D. Expression of chemokines on the surface of different human endothelia. *Immunol Cell Biol* 2005; 83: 375–382.
- 12 Takaoka A, Hayakawa S, Yanai H, *et al.* Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. *Nature* 2003; 424: 516–523.
- 13 Spurrell JC, Wiehler S, Zaheer RS, *et al.* Human airway epithelial cells produce IP-10 (CXCL10) *in vitro* and *in vivo* upon rhinovirus infection. *Am J Physiol Lung Cell Mol Physiol* 2005; 289: L85–L95.

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