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a significant burden of morbidity and mortality as well as substantial healthcare costs (6). There are currently no e□ective therapeutics, or preventative vaccines, licensed for use to treat or prevent HRV infection.

(Thermo-Fisher, UK) and seeded at 5 × 10^4 cells/ml for 12-well plates and allowed to grow for 24 h before infection or

A qPCR Primer sequences utilized in this study.

	_●	*v*	**n**
PADI1	TCTACTCGGACTGGC TCTCTG	TGCTTCTTTTTGCCTG GTGTTT	This work
PADI2	GCTTTCCTCTGTCTG GTGGT	TTTCTTTGTGCCGGG GATGG	This work
PADI3	CTGCAGAGAATCGTG CGTGT	TGATCTCCAAAGTCG CGTCAA	This work
PADI4	CCATCCTGCTGGTGA ACTGT	GAAGTCCTTGGGGGT CTTCG	This work
GAPDH	AAGCTCATTTCCTGG TATGACA	TCTTACTCCTTGGAG GCCATGT	(

induced an increase in CCL5 secretion compared to untreated cells, while the addition of LL-37 reduced this e□ect. The e□ects of LL-37$_{1cit}$ were indistinguishable from those of native LL-37, whereas LL-37$_{2cit,}$ and LL-37$_{3cit}$ showed a moderate loss in their ability to reduce HRV-induced CCL5 secretion. LL-37

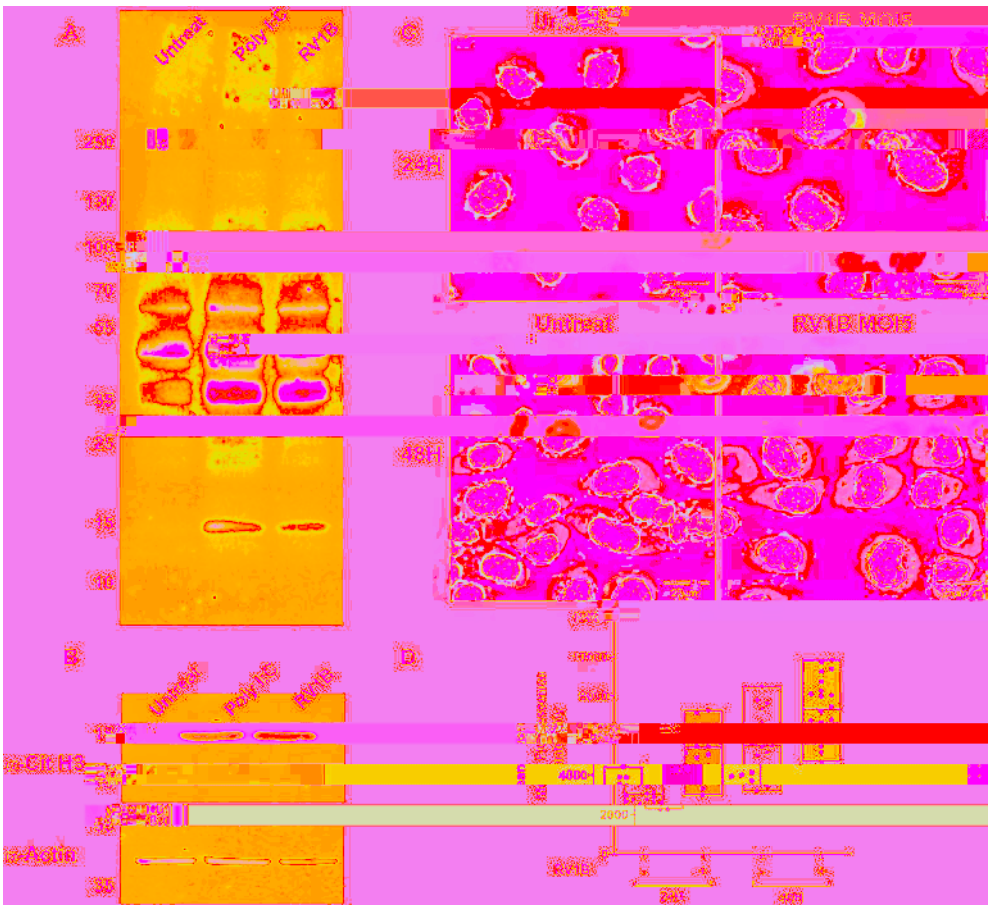
Human rhinovirus and Poly I:C stimulation increase PAD2 protein expression in lung epithelial cells. Human bronchial epithelial (16HBE14$^{\circ-}$) cells were infected with HRV1B MOI = 5 for 6, 24 and 48 h before mRNA levels of PADI2 **A** and PADI4 were assessed by qPCR. Poly I:C was used as positive control for viral dsRNA. Protein levels of PAD2 and PAD4 at 24 or 48 h after RV1B infection were measured by FACS (PAD2 and PAD 4) and Western immunoblotting (PAD 2 only, 24 h post infection). UV irradiated virus (UV-RV1B) was used as replication deficient virus control. Values represent fold change expression over untreated cells (dotted line) with data representing the mean ± SEM of 4 different experiments. Representative histogram plots for PAD2 or PAD4 protein expression in 16HBE14$^{\circ-}$ are shown. A representative Western immunoblot (representative of $n = 3$) together with quantification by densitometry are displayed , showing enhanced PAD2 protein in 16HBE14$^{\circ-}$ cells after 24 h of RV1B infection at MOI = 5. Confocal microscopy images show enhanced PAD2 staining (red) in 16HBE14$^{\circ-}$ cells after 48 h of RV1B infection at MOI = 1 or MOI = 5 . Statistical analysis in **A** was performed on CT values via a two-way ANOVA with Tukey multiple comparisons test. $^{\$}p \leq 0.05$, $^{\$\$}p \leq 0.01$ denoting significance on Poly I:C treatment vs. untreated (24 and 48 h, respectively). $^{*}p \leq 0.05$, $^{**}p \leq 0.01$.

staining and protein citrullination was detectable in the presence of HRV (F r C D). Taken collectively these data suggest that HRV infection, or activation of TLR3, results in increased protein citrullination, including histone H3.

Previous studies have shown PAD2 and PAD4 isoforms citrullinate LL-37 peptides in vitro, inhibiting the direct antibacterial activity of LL-37 (30), the ability of LL-37 to enhance nucleic acid uptake and subsequent sensing by plasmacytoid dendritic cells (53), and the ability of LL-37 to reduce inflammation in response to lipopolysaccharide (LPS) (35). Our results confirm that citrullination abrogates the antimicrobial and immunomodulatory activities of LL-37 in the context of viral infection, and further show that citrullination of LL-37 abrogates its antiviral activity, identifying a novel role for citrullination in the innate response to viral infection.

Airway epithelial cells are the main target of HRV infection and replication. In response to infection, these cells can release host defense peptides (11) which play a key role in the inflammatory and innate immune responses (12). LL-37 concentrations of ∼5 μg/ml have been recovered in the bronchoalveolar lavage fluid (BAL) from uninfected infants, with levels up to ∼30 μg/ml post infection (11, 54). Here we used concentrations of exogenous LL-37 that mimic physiological concentrations, and in agreement with our own recent studies (36) and others (37, 38), show that direct incubation of LL-37 with HRV prior to infection of lung epithelial cells is e ective at reducing HRV RNA copy number and virion release into cell supernatants.

4 Human rhinovirus and Poly I:C stimulation increase PAD2 expression in $CD14^{-}CD16^{++}$ PBMCs. Peripheral blood mononuclear cells (PBMC) were isolated and exposed to different doses of HRV (viral MOI of 1 or 5) or to 10 μg/ml of Poly I:C for 24 or 48 h. Intracellular PAD2 expression was assessed in different PBMC subsets by FACS. Dot plots indicate the gating strategy used and histogram overlays indicate representative PAD2 levels (MFI) in different PBMCs subsets, **A** $CD3^{+}$ $CD56^{-}$ T-cells, $CD19^{+}$ $CD3^{-}$ $CD56^{-}$ B-cells, and $CD14^{+}$ $CD16^{low}$ $CD19^{-}$ $CD3^{-}$ $CD56^{-}$ Monocytes. Bars represent the Mean Fluorescence Index (MFI) of PAD2 levels expressed in each PBMC subset after 48 h of infection: T-cells, NK-T cells, NK-cells, B-cells, $CD14^{-}$ $CD16^{++}$ monocytes, and $CD14^{+}$ $CD16^{low}$ monocytes. Bars indicate the mean ±



Human rhinovirus and Poly I:C stimulation increase protein citrullination in 16HBE14° cells. Human bronchial epithelial (16HBE14°) cells were infected with HRV1B MOI = 5 or treated with Poly IC and total citrullination was measured with a monoclonal antibody against peptidyl citrulline (F95). **A** After 24 h, lysates were obtained and blotted with F95 antibody, α-citrullinated histone H3, and actin, which was used as loading control. Confocal microscopy images were also taken at 24 or 48 h after infection, showing F95 staining (RED) and DAPI as nuclear counterstaining (BLUE). Quantification of at least four different fields of view from is shown in , with each dot representing a different cell and boxes displaying min to max and mean values of F95 staining intensity.

the net charge of LL-37 peptide, resulting in LL-37$_{5cit}$ with a +1 net charge, as opposed to the +6 net charge of native LL-37 (30). This would a□ect the ability of LL-37 to interact electrostatically with negatively charged molecules such as LPS (56). Interestingly, while citrullination of LL-37 substantially reduced the antibacterial activity of the peptide against S. aureus, prior studies have determined that proteinases released by this

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D r The findings and conclusions in this report are those of the authors and do not necessarily represent the o□cial position of CDC.

Confl o n r The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as-64.75528-19,1(a