Imiquimod shows anti-viral actions in human bronchial epithelium - implications for COVID-19 treatment

Tillgren, Sofia; Nieto Fontarigo, Juan José; Cerps, Samuel; Hvidtfeldt, Morten; Sverrild, Asger; Porsbjerg, Celeste; Uller, Lena

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Imiquimod improves viral resistance and tolerance in human asthmatic bronchial epithelium

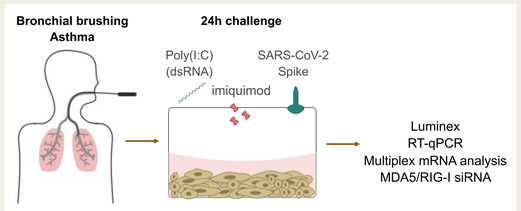
SOFIA TILLGREN[1], JUAN JOSÉ NIETO FONTARIGO[1], SAMUEL CERPS[1], MORTEN HVIDTFELDT[2], ASGER SVERRILD[2], CELESTE PORSBJERG[2], LENA ULLER[1]

[1] Respiratory Immunopharmacology, Department of Experimental Medical Science, Lund University, Sweden
[2] Department of Pulmonary Medicine, Bispebjerg Hospital, Copenhagen University, Denmark

Background

- Bronchial epithelial cells (HBECs) are main targets of respiratory viral infections responsible for important anti-viral and inflammatory responses
- Asthmatic patients may have dysregulated epithelial mechanisms involved in viral resistance (affecting level of infection) as well as infection tolerance (affecting level of inflammation) at viral infections
- Drugs that boost viral resistance and increase infection tolerance are of interest for treating airway infections caused by virus including SARS-CoV-2
- We hypothesized that the TLR7 agonist imiquimod (imq) may combine these desired treatment actions in human bronchial epithelial cells

Methods



HBECs from asthmatic donors (N=18) were treated with imq alone or in combination with the viral mimic poly (I:C) or the SARS-CoV-2 spike protein 1 (SP1) for 24 hours to study effects on viral resistance and tolerance. siRNA against MDA5/RIG-I was used to investigate involvement of cytosolic receptors in these responses. Anti-viral and pro-inflammatory mediators were analyzed by Luminex, RT-qPCR and mRNA multiplex analysis (Nanostring).

Results

I. *Viral resistance:* Imq increases IFN-β expression in bronchial epithelial cells challenged with SARS-CoV-2 spike protein or poly(I:C)

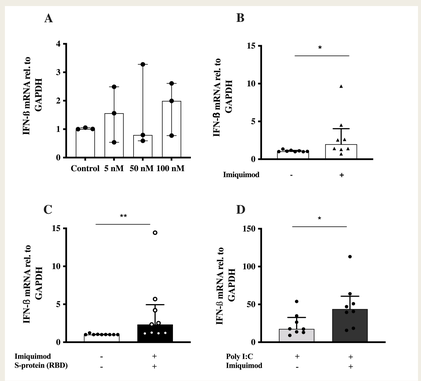


Figure 1. Effect of imq and SP1 treatment on IFN-β expression in human bronchial epithelial cells. IFN-β gene expression in bronchial epithelial cells post SP1 alone (A) imq alone (B) SP1 (50 nM) and imq (C) or poly(I:C) and imq (D) treatment. Gene expression was measured by RT-qPCR. Data are expressed as fold change against untreated cells. * $p < 0.05$; ** $p < 0.01$, Wilcoxon Signed Rank Test, N = 3-9

II. MDA5 and RIG-I are involved in imq-mediated induction of IFN-β expression

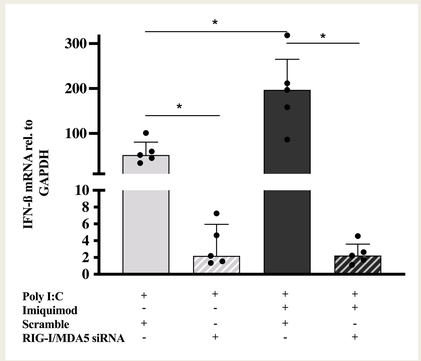


Figure 2. siRNA knock-down of MDA5 and RIG-I in bronchial epithelial cells. RT-qPCR analysis of IFN-β expression during siRNA mediated knock-down of MDA5 and RIG-I, data are normalized to untreated cells. * $p < 0.05$; ** $p < 0.01$, RM one-way ANOVA. N = 5.

III. *Viral resistance:* Imq reduces ACE2 expression in bronchial epithelial cells challenged with SARS-CoV-2 spike protein or poly(I:C)

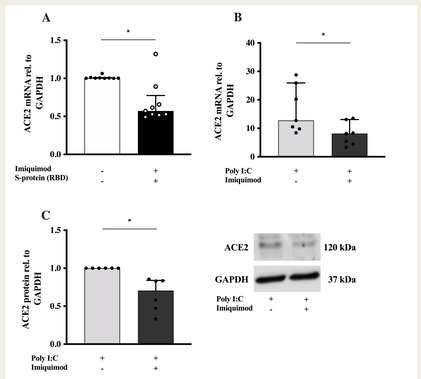


Figure 3. Effect of imq treatment on ACE2 expression in human bronchial epithelial cells. ACE2 gene expression in cells stimulated with SARS-CoV-2 spike protein (A) or poly(I:C) (B), during treatment with imq. Gene expression was measured by RT-qPCR, and data are expressed as fold change against untreated cells. Protein expression of ACE2, as measured by western blot (C), data are normalized to poly(I:C) stimulated cells.* $p < 0.05$; ** $p < 0.01$, Wilcoxon Signed Rank Test, N = 6-9.

IV. *Viral tolerance:* Imq decreases poly(I:C)-induced cytokines in bronchial epithelial cells

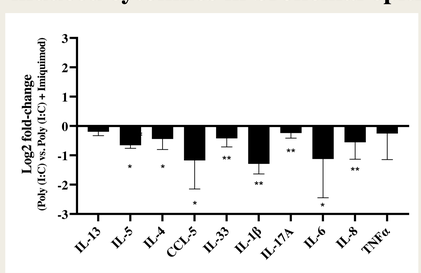


Figure 4. Imq impact on epithelial-derived cytokine release in poly(I:C) stimulated cells. Cytokine release in poly(I:C) and poly(I:C) + imiquimod treated cells was measured using multiplex ELISA (Luminex), data are normalized to poly(I:C) and expressed as log2 fold change. * $p < 0.05$; ** $p < 0.01$, Wilcoxon Signed Rank Test. N = 8.

V. Multiplex mRNA analysis reveals a role of SIGIRR and C1QBP in the imq-mediated effects on bronchial epithelium

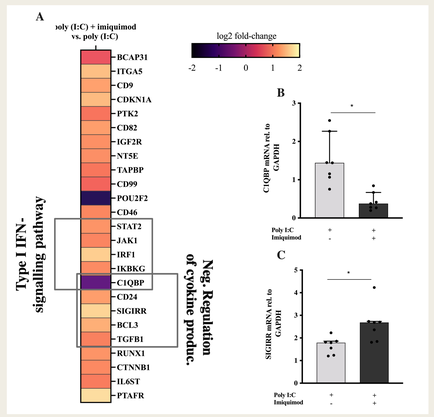


Figure 5. mRNA pathway analysis of poly(I:C) and imq treated human bronchial epithelial cells. Multiplex mRNA analysis of poly(I:C) and poly(I:C) + imq treated cells (A), graph is showing significant (adj $p<0.05$) differentially expressed genes in poly(I:C) compared to poly(I:C) and imq treated cells. Confirmation of C1QBP (B) downregulation and SIGIRR (C) upregulation by RT-qPCR, expressed as fold change compared to untreated cells. * $p < 0.05$; ** $p < 0.01$, Multiple t-test analyses with the corrected method of Benjamini and Yekutieli (A), Wilcoxon Signed Rank Test (B-C), N = 3 (A), 7 (B-C).

Conclusions

- Imiquimod increases IFN-β expression in bronchial epithelial cells, possibly involving up-regulation of the cytosolic receptors MDA5 and RIG-I. C1QBP, which negatively modulates MDA5 and RIG-I, was decreased
- Imiquimod decreases expression of the SARS-CoV-2 entry receptor ACE2
- Imiquimod decreases poly(I:C)-induced inflammatory cytokines. SIGIRR, a negative regulator of cytokine signaling, might play a role in this action
- Our findings highlight a possibility of developing drugs suited for anti-viral airway treatment by combining improved viral resistance with improved tolerance to viral infection

Contact

Sofia Tillgren, MSC, PhD-student
Respiratory Immunopharmacology, Department of Experimental Medical Science, Lund University, Sweden
Sofia.tillgren@med.lu.se

Vetenskapsrådet
Hjärt-Lungfonden