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

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Imiquimod improves viral resistance and tolerance in human asthmatic bronchial epithelium

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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 viruses 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 (Nanosting).

Results

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

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 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 C1QB 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 C1QB (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 Benjamin 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. C1QB, 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

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