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Glucagon-like peptide-1 receptor signaling attenuates RSV-induced type 2 responses and immunopathology

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Capsule Summary

GLP-1R signaling, an emerging anti-inflammatory therapeutic target, attenuated type 2-associated immunopathology in mice infected with a strain of RSV that was isolated from a hospitalized infant with severe lower respiratory tract infection and bronchiolitis.

Keywords

glucagon-like peptide-1 (GLP-1); respiratory syncytial virus (RSV); group 2 innate lymphoid cells (ILC2); IL-13; IL-33; type 2 immunity (Th2); phenome-wide association study (PheWAS)

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To the editor

Glucagon-like peptide-1 receptor (GLP-1R) agonists, which potentiate insulin and suppress glucagon secretion, are a well-accepted and safe treatment for Type II diabetes.¹ Although GLP-1R agonists are currently used for their ability to potentiate insulin and suppress glucagon secretion, recent evidence suggests that GLP-1R signaling also has anti-inflammatory effects.²⁻⁴ Severe RSV-associated illness is in part caused by IL-13 production, which mediates the mucus production that directly contributes to airway obstruction and respiratory failure.⁵ We hypothesized that GLP-1R signaling inhibits IL-13-mediated immunopathology of RSV 12/12-6, a strain of RSV that was isolated from a hospitalized infant with severe lower respiratory tract infection and bronchiolitis.

Eight week old mice were infected with 9×10^5 PFU of RSV. RSV 12/12-6 induced significant lung IL-13 and airway mucus, mimicking what is seen in patients with severe infection (Fig E1). We administered GLP-1R agonist or vehicle (0.1% BSA in PBS) twice daily beginning 2 days prior to RSV infection until all endpoints (Fig E2). GLP-1R agonist treatment significantly decreased lung IL-13 protein expression compared to vehicle treatment in RSV-infected mice (Fig 1, A). We identified the cellular sources of IL-13 that GLP-1R signaling was inhibiting. GLP-1R agonist treatment in RSV-infected mice significantly decreased the total number of cells in the lung, the total number of group 2 innate lymphoid cells (ILC2), and the percentage of ILC that were IL-13⁺ compared to RSV-infected vehicle-treated mice (Fig E3A, Fig E4A&F & Fig 1, B&E). There was significantly decreased MFI of IL-13 and CD127 on the ILC2 of RSV-infected GLP-1R agonist-treated mice compared to RSV-infected vehicle-treated mice, indicating decreased IL-13 production and CD127 expression on a per ILC2 basis with GLP-1R agonist treatment (Fig E4B-E). GLP-1R agonist treatment in RSV-infected mice significantly decreased the numbers of CD4⁺ T cells and basophils, as well as IL-13⁺ Th2 cells and basophils compared to RSV-infected vehicle-treated mice (Fig E3B-C, Fig E4G-H & Fig 1, C-D).

Moreover, there were significant decreases in methacholine-induced airway responsiveness and mucus severity scores in RSV-infected GLP-1R agonist-treated mice compared to RSV-infected vehicle-treated mice (Fig 1, F-H). RSV-infected GLP-1R agonist-treated mice had significantly decreased numbers of total bronchoalveolar lavage (BAL) cells and lymphocytes compared to RSV-infected vehicle-treated mice (Fig 1, J). Administration of the GLP-1R agonist beginning 2 days after RSV infection also significantly decreased lung IL-13 and there was a trend towards decreased airway mucus (Fig E5). Collectively, these data demonstrate that GLP-1R signaling attenuates IL-13-mediated immunopathology during RSV infection.

IL-33 activates type 2 cytokine-producing immune cells including ILC2, Th2, and basophils. We used *Il33^{Citrine/+}* reporter mice to examine the effect of GLP-1R signaling on IL-33 expression on a per epithelial cell basis. GLP-1R agonist treatment in RSV-infected mice significantly decreased the total number of cells in the lung, the total number of IL-33-expressing epithelial cells, and the percentage of epithelial cells that were IL-33⁺ compared to RSV-infected vehicle-treated mice (Fig E6A-B & Fig 2, A). There was significantly decreased MFI of IL-33 in the epithelial cells of RSV-infected GLP-1R agonist-treated mice

compared to RSV-infected vehicle-treated mice, indicating decreased IL-33 expression on a per epithelial cell basis with GLP-1R agonist treatment (Fig 2, B–C). RSV-infected GLP-1R agonist-treated mice had significantly decreased lung IL-33 protein expression compared to RSV-infected vehicle-treated mice (Fig 2, D). These data indicate that GLP-1R agonist treatment inhibits the expression of IL-33 by epithelial cells during RSV infection.

To determine whether GLP-1R agonist treatment had a deleterious effect on viral-associated disease severity parameters, we evaluated viral load, an indicator of RSV disease severity.⁶ There were no significant differences in the viral load between RSV-infected GLP-1R agonist and vehicle-treated mice (Fig 2, E). Consistent with these data, we did not observe any significant differences in lung interferon- γ (IFN- γ) expression or IFN- γ ⁺ Th1 and natural killer (NK) cells between RSV-infected GLP-1R agonist and vehicle-treated mice 6 days post-infection (Fig E4B&D, Fig 2, F & Fig E7A–B). Further, there were no significant differences in lung IFN- α , IFN- β , or IL-27 protein expression between RSV-infected GLP-1R agonist and vehicle-treated mice (Fig E7C–E). We also found that there were no statistically significant changes in plasma glucose or insulin levels with GLP-1R treatment compared to vehicle (Fig E7F & G).

To determine whether GLP-1R agonist treatment during primary infection has an impact on the immune response to a later secondary infection, we infected mice with RSV a second time following primary RSV infection (Fig E8). GLP-1R agonist treatment during primary infection significantly decreased the number of RSV-induced total BAL cells and lymphocytes compared to vehicle treatment after secondary infection (Fig E9A). The mice treated with GLP-1R agonist during primary infection did not exhibit altered lung IFN- γ expression nor RSV F-protein-specific antibody responses compared to vehicle-treated mice during secondary RSV infection (Fig E9B–E). These data demonstrate that GLP-1R agonist treatment does not exacerbate disease or impede anti-viral responses.

We next sought to identify associations between GLP-1 signaling and human RSV disease. The phenome-wide association study (PheWAS) is a new, validated reverse genetics approach that associates genetic variants of interest with phenotypes by linking a database of de-identified genotyping to a broad range of electronic medical record (EMR)-derived clinical phenotypes. The EMR phenotypes are derived from cluster of common *International Classification of Diseases, Ninth Revision*, codes. The loss-of-function rs7578597 variant of *THADA*, encoding thyroid adenoma-associated protein, is associated with lower beta-cell response to GLP-1.⁷ A PheWAS on the single nucleotide polymorphism (SNP) rs7578597 (missense, T1187A) and 1,000 phenotypes from the Vanderbilt BioVU biobank of 29,713 individuals of European ancestry (EA)⁸ revealed a highly significant association of rs7578597 with acute bronchitis and bronchiolitis (OR = 1.24, P = 6.3×10^{-3} ; Fig 2, G and Table E1).

This study is the first investigation of GLP-1R signaling during viral infection. We show that administration of a GLP-1R agonist attenuates type 2-associated immunopathology during RSV infection. This is also the first report of an FDA-approved pharmacologic agent inhibiting lung IL-33 protein expression, and this finding has significant implications as it may provide an alternative to biologic therapies such as monoclonal antibodies or receptor

antagonists that target IL-33-mediated diseases.⁹ Together, these data highlight a novel potential therapeutic for RSV infection, a disease for which there currently is no treatment after infection has occurred.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ILC2	group 2 innate lymphoid cells
RSV	respiratory syncytial virus
Th2	T helper 2
GLP-1	glucagon-like peptide-1
WT	wild type
MFI	mean fluorescence intensity
PFU	plaque forming unit
PheWAS	phenome-wide association study

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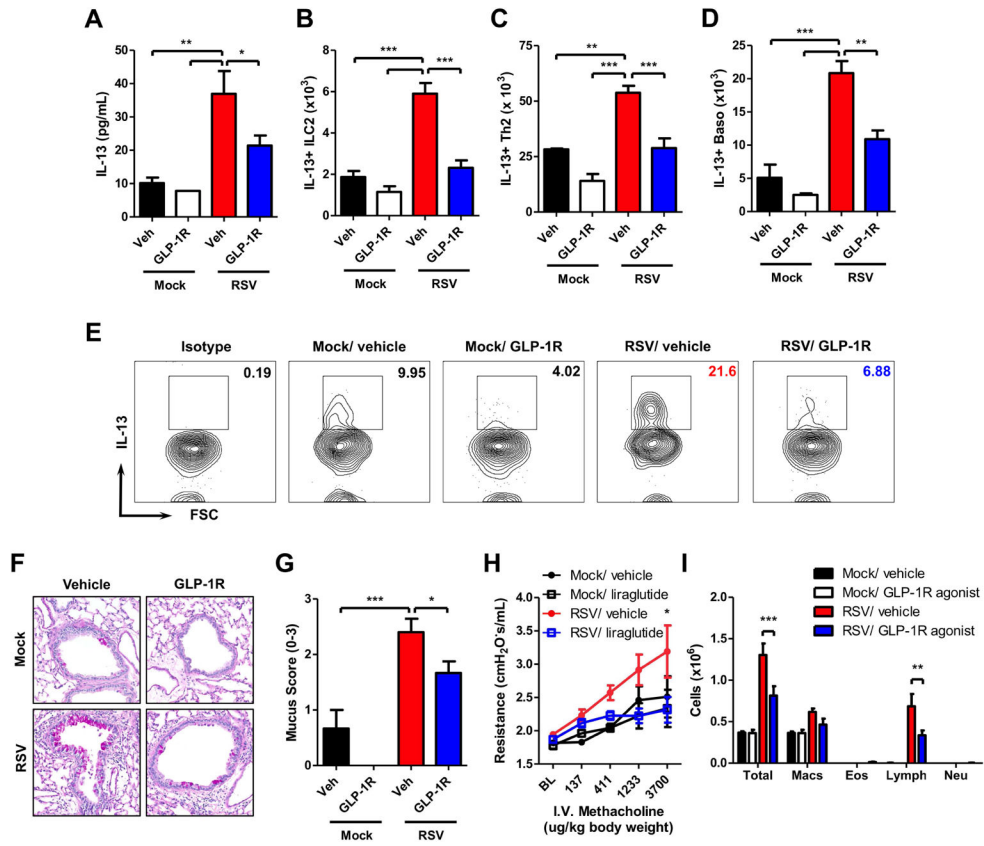


Figure 1. GLP-1R agonist decreases RSV-induced type 2 responses and immunopathology (A) ELISA for IL-13 in whole lung homogenate (right lung only). (B) Total number of IL-13⁺ ILC2, (C) Th2 cells, and (D) basophils. (E) Representative IL-13 expression measured by flow cytometry in ILC2. (F) Representative PAS-stained section of mucus-containing airways in the lungs (40x magnification); arrowhead denotes intraluminal mucus strand. (G) Quantification of airway mucus from the experiment in A. (H) Airway responsiveness and (I) BAL cell counts. Data plotted as mean + SEM. n = 3–6 mice per group representative of 3 (A) or 2 (B–G & I) independent experiments. n = 6–12 mice per group combined from 2 independent experiments (H). *p < 0.05, **p < 0.01, ***p < 0.001 by one-way (B & E–H) or two-way (C–D) ANOVA. BL = baseline.

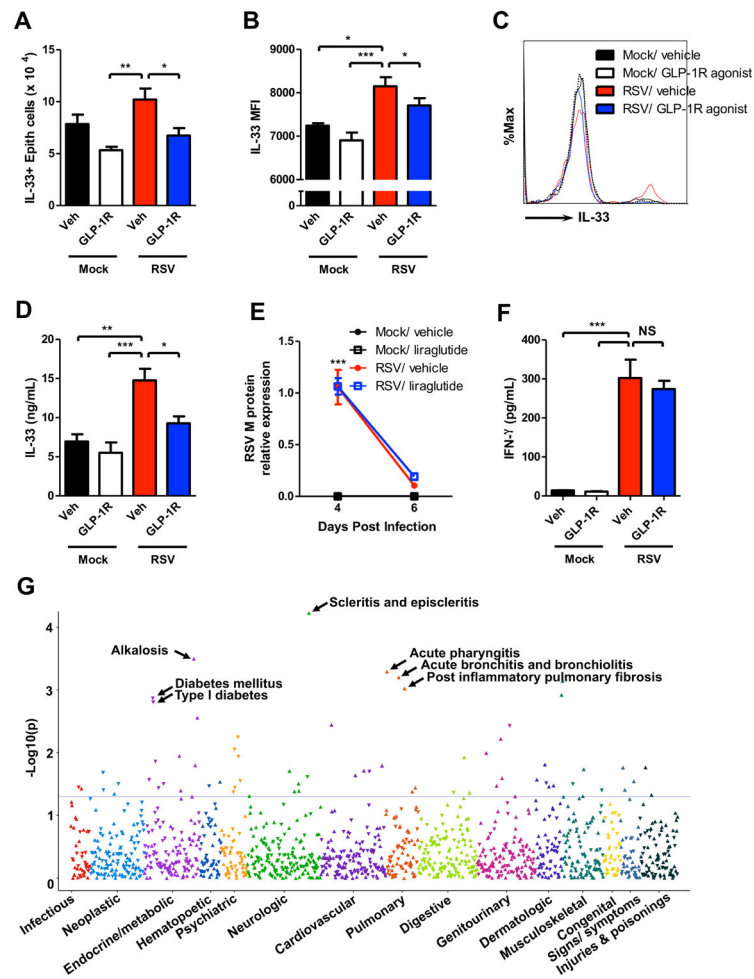


Figure 2. GLP-1R signaling decreases IL-33, does not increase viral titer or decrease IFN- γ production, and associates with acute bronchiolitis in humans

(A) Total number of IL-33⁺ epithelial cells, (B) MFI of IL-33 expression in epithelial cells, (C) representative IL-33 expression measured by flow cytometry in epithelial cells, and (D) ELISA for IL-33 in whole lung homogenate (left lung only). (E) Lung mRNA RSV M protein expression normalized to GAPDH. (F) ELISA for IFN- γ in whole lung homogenate (right lung only). (G) Phenome-wide association study (PheWAS) plot for THADA rs7578597 using logistic regression assuming an additive genetic model adjusted for age, sex, study site, and the first 3 principal components. rs7578597 associated with acute bronchiolitis (OR = 1.24, $P = 6.3 \times 10^{-3}$). Data plotted as mean + SEM. $n = 3-6$ mice per group representative of 2 independent experiments (A–F). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by one-way (A–B, D, & F) or two-way (E) ANOVA. NS = not significant.