

effect of a TBXAS inhibitor (picotamid) on the IMQ-induced psoriasis model. Mice treated with the TBXAS inhibitor exhibited significantly reduced ear-swelling responses accompanied with decreased IL-17A production from $V\gamma 4^+ \gamma \delta$ T cells (Fig 2, F and G). Collectively, these results suggest that TxA_2 -TP signaling facilitates IL-17A production from $V\gamma 4^+ \gamma \delta$ T cells and promotes psoriatic dermatitis.

In this study, using a lipidomics approach, we identified an upregulation of TxA_2 in the psoriatic lesions. We have also clarified TxA_2 as a proinflammatory mediator in psoriatic dermatitis by means of TP-deficient mice and pharmacologic approaches. It has been well known that TxA_2 is a potent activator of platelet aggregation and smooth muscle contraction¹; however, the role of TxA_2 in innate immune responses remains largely unknown. Of note, TxA_2 was once reported as a negative regulator rather than a positive regulator in acquired immune responses.⁷ Our results, therefore, revealed an unexpected proinflammatory role of TxA_2 in IL-17-mediated psoriatic dermatitis.

There are several unsolved issues in our study. First, the source of TxA_2 remained unclear. Cutaneous DCs may be a candidate population, because DCs exhibited higher mRNA expression of *Tbxas* compared with $\gamma \delta$ T cells, and activated DCs produce abundant TxA_2 .⁷ Therefore, TxA_2 from cutaneous DCs that are physically associated with dermal $\gamma \delta$ T cells in the skin⁹ may stimulate TP on $\gamma \delta$ T cells and facilitate the IL-17A production. Second, it remains unknown whether TP signaling contributes to the development of human psoriasis, because human psoriasis is a T_H17 -driven disease, and the role of $\gamma \delta$ T cells in human psoriasis may be at best ancillary.² Although human relevance should be further examined, blockade of TP signaling may offer a new therapeutic strategy that enables improved treatment of psoriasis.

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Glucagon-like peptide 1 receptor signaling attenuates respiratory syncytial virus-induced type 2 responses and immunopathology



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 used currently for their endocrine properties, recent evidence suggests that GLP-1R signaling also has anti-inflammatory effects.²⁻⁴ Severe respiratory syncytial virus (RSV)-associated illness is in part caused by IL-13 production, which mediates the mucus metaplasia 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 plaque-forming units (PFU) of RSV. RSV 12/12-6 induced significant lung IL-13 and airway mucus, mimicking what is seen in patients with severe infection (see Fig E1 in this article's Online Repository at www.jacionline.org). We administered GLP-1R agonist or vehicle (0.1% BSA in PBS) twice daily beginning 2 days before RSV infection until all end points were reached (see Fig E2 in this article's Online Repository at www.jacionline.org). GLP-1R agonist treatment significantly decreased lung IL-13 protein expression compared with vehicle treatment in RSV-infected mice (Fig 1, A). We identified the

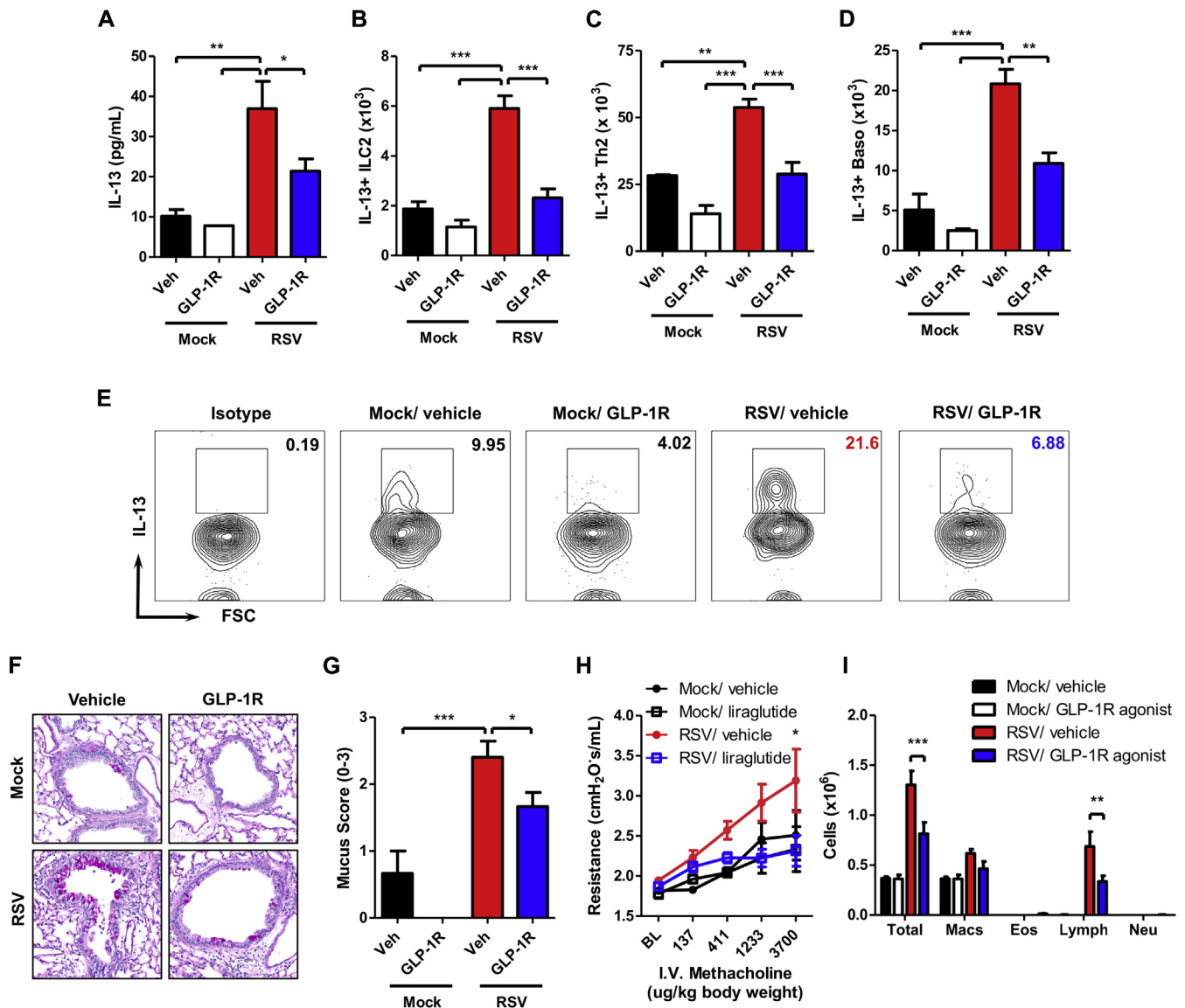


FIG 1. GLP-1R agonist decreases RSV-induced type 2 responses and immunopathology. **A**, ELISA for IL-13 in whole-lung homogenate (right lung only). **B–D**, Total number of IL-13⁺ ILC2s (Fig 1, **B**), T_H2 cells (Fig 1, **C**), and basophils (Fig 1, **D**). **E**, Representative IL-13 expression measured by using flow cytometry in ILC2s. **F**, Representative PAS-stained section of mucus-containing airways in the lungs (×40 magnification). Arrowhead denotes intraluminal mucus strand. **G**, Quantification of airway mucus from the experiment in Fig 1, **A**. **H** and **I**, Airway responsiveness (Fig 1, **H**) and BAL fluid cell counts (Fig 1, **I**). *Eos*, Eosinophils; *Lymph*, lymphocytes; *Macs*, macrophages; *Neu*, neutrophils. Data are plotted as means + SEMs. N = 3 to 6 mice per group representative of 3 (Fig 1, **A**) or 2 (Fig 1, **B–G** and **I**) independent experiments. n = 6 to 12 mice per group combined from 2 independent experiments (Fig 1, **H**). **P* < .05, ***P* < .01, and ****P* < .001, 1-way (Fig 1, **B** and **E–H**) or 2-way (Fig 1, **C** and **D**) ANOVA. BL, Baseline.

cellular sources of IL-13 inhibited by GLP-1R signaling. GLP-1R agonist treatment in RSV-infected mice significantly decreased total numbers of cells in the lungs, total numbers of group 2 innate lymphoid cells (ILC2s), and percentages of IL-13⁺ innate lymphoid cells (ILCs) compared with those in RSV-infected vehicle-treated mice (Fig 1, **B** and **E**, and see Fig E3, **A**, and Fig E4, **A** and **F** in this article's Online Repository at www.jacionline.org). There was significantly decreased mean fluorescence intensity (MFI) of IL-13 and CD127 on the ILC2s of RSV-infected GLP-1R agonist-treated mice compared with RSV-infected vehicle-treated mice, indicating decreased IL-13

production and CD127 expression on a per-ILC2 basis with GLP-1R agonist treatment (see Fig E4, **B–E**). GLP-1R agonist treatment in RSV-infected mice significantly decreased numbers of CD4⁺ T cells and basophils, as well as IL-13⁺ T_H2 cells and basophils, compared with RSV-infected vehicle-treated mice (Fig 1, **C** and **D**, and see Fig E3, **B** and **C**, and Fig E4, **G** and **H**).

Moreover, there were significant decreases in methacholine-induced airway responsiveness and mucus severity scores in RSV-infected GLP-1R agonist-treated mice compared with RSV-infected vehicle-treated mice (Fig 1, **F–H**). RSV-infected

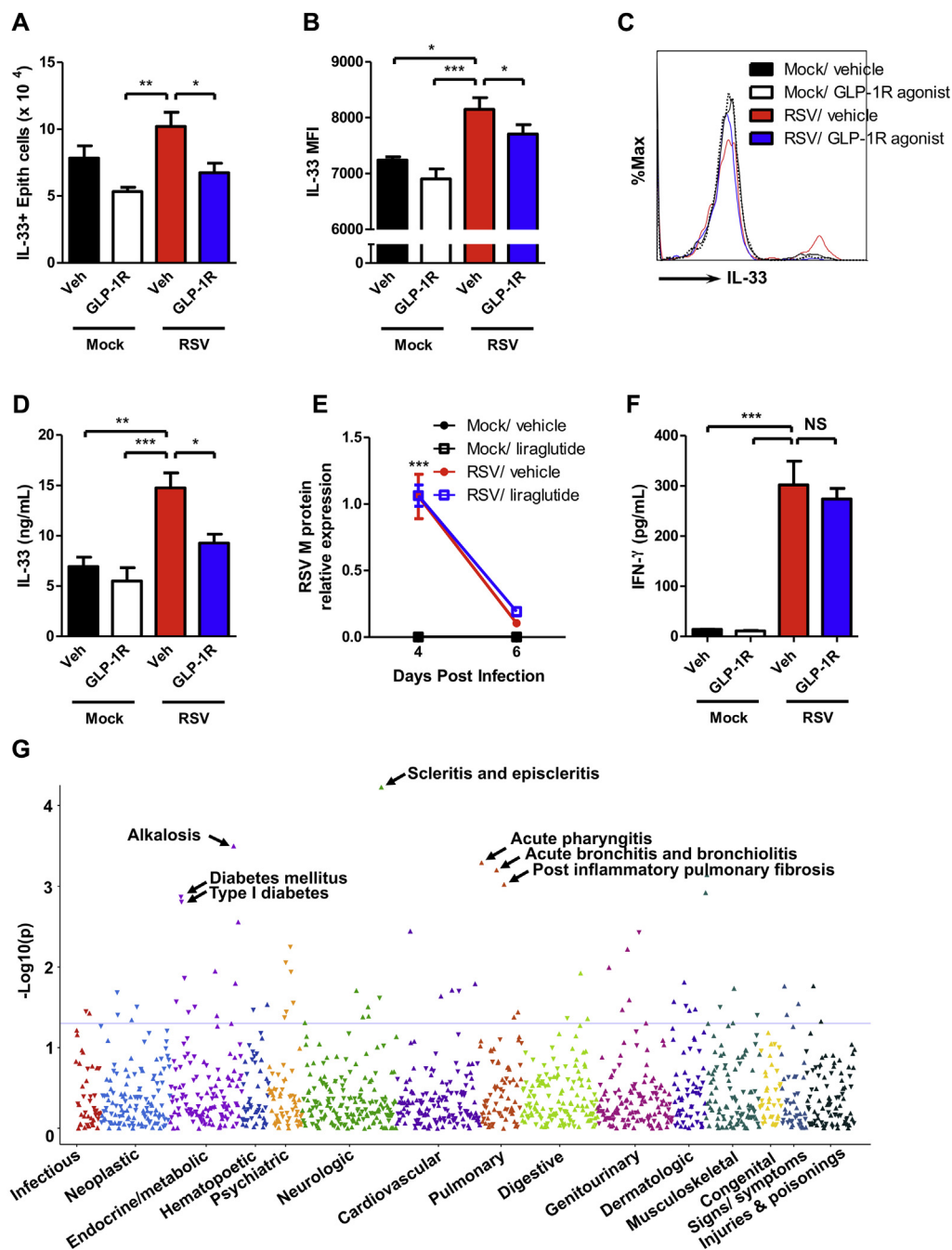


FIG 2. GLP-1R signaling decreases IL-33 levels, does not increase viral titer or decrease IFN- γ production, and associates with acute bronchiolitis in human subjects. **A**, Total number of IL-33⁺ epithelial cells. **B**, MFI of IL-33 expression in epithelial cells. **C**, Representative IL-33 expression measured by using flow cytometry in epithelial cells. **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**, 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 is shown (odds ratio, 1.24; $P = 6.3 \times 10^{-3}$). Data were plotted as means $\pm$ SEMs. $n = 3$ -6 mice per group representative of 2 independent experiments. * $P < .05$, ** $P < .01$, and *** $P < .001$, 1-way (Fig 2, A, B, D, and F) or 2-way (Fig 2, E) ANOVA. NS, Not significant.

GLP-1R agonist-treated mice had significantly decreased numbers of total bronchoalveolar lavage (BAL) cells and lymphocytes compared with RSV-infected vehicle-treated mice (Fig 1, I). Administration of the GLP-1R agonist beginning

2 days after RSV infection also significantly decreased lung IL-13 levels, and there was a trend toward decreased airway mucus (see Fig E5 in this article's Online Repository at www.jacionline.org). 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 ILC2s, T_H2 cells, 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 total numbers of cells in the lung, total numbers of IL-33–expressing epithelial cells, and percentages of IL-33⁺ epithelial cells compared with those in RSV-infected vehicle-treated mice (Fig 2, A, and see Fig E6, A and B). There was significantly decreased MFI of IL-33 levels in the epithelial cells of RSV-infected GLP-1R agonist–treated mice compared with RSV-infected vehicle-treated mice, indicating decreased IL-33 expression on a per-epithelial-cell basis with GLP-1R agonist treatment (Fig 2, B and C). RSV-infected GLP-1R agonist–treated mice had significantly decreased lung IL-33 protein expression compared with RSV-infected vehicle-treated mice (Fig 2, D). These data indicate that GLP-1R agonist treatment inhibits expression of IL-33 by epithelial cells during RSV infection.

To determine whether GLP-1R agonist treatment had a deleterious effect on virus-associated disease severity parameters, we evaluated viral load, an indicator of RSV disease severity.⁶ There were no significant differences in viral load between RSV-infected GLP-1R agonist–treated and vehicle-treated mice (Fig 2, E). Consistent with these data, we did not observe any significant differences in lung IFN- γ expression or IFN- γ ⁺ T_H1 and natural killer (NK) cells between RSV-infected GLP-1R agonist– and vehicle-treated mice 6 days after infection (Fig 2, F, and see Fig E4, B and D, and Fig E7, A and B, in this article's Online Repository at www.jacionline.org). Furthermore, 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 (see Fig E7, C–E). We also found that there were no statistically significant changes in plasma glucose or insulin levels with GLP-1R treatment compared with vehicle treatment (Fig E7, F and G).

To determine whether GLP-1R agonist treatment during primary infection has an effect on the immune response to a later secondary infection, we infected mice with RSV a second time after primary RSV infection (see Fig E8 in this article's Online Repository at www.jacionline.org). GLP-1R agonist treatment during primary infection significantly decreased the number of RSV-induced total BAL fluid cells and lymphocytes compared with vehicle treatment after secondary infection (see Fig E9, A, in this article's Online Repository at www.jacionline.org). Mice treated with GLP-1R agonist during primary infection did not exhibit altered lung IFN- γ expression or RSV F-protein–specific antibody responses compared with vehicle-treated mice during secondary RSV infection (see Fig E9, B–E). These data demonstrate that GLP-1R agonist treatment does not exacerbate disease or impede antiviral 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 deidentified genotyping to a broad range of electronic medical record (EMR)–derived clinical phenotypes. The EMR phenotypes are derived from a cluster of common International Classification of Diseases, Ninth

Revision, codes. The loss-of-function rs7578597 variant of *THADA*, which encoded thyroid adenoma-associated protein, is associated with lower β 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 subjects of European ancestry⁸ revealed a highly significant association of rs7578597 with acute bronchitis and bronchiolitis (odds ratio, 1.24; $P = 6.3 \times 10^{-3}$; Fig 2, G, and see Table E1 in this article's Online Repository at www.jacionline.org).

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 a US Food and Drug Administration–approved pharmacologic agent inhibiting lung IL-33 protein expression, and this finding has significant implications because it can provide an alternative to biologic therapies, such as mAbs or receptor antagonists that target IL-33–mediated diseases.⁹ Together, these data highlight a novel potential therapeutic agent for RSV infection, a disease for which there is currently no treatment after infection has occurred.

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Severity of allergic rhinitis assessed by using urine metabolomic profiling: Proof of concept



To the Editor:

The ability to monitor the severity of respiratory diseases is difficult in the typical clinical setting, particularly for diseases of the upper airway, such as allergic rhinitis (AR). Patients often do not recognize how severe their symptoms are because of progressive “tolerizing” to symptoms. Chronic nasal congestion becomes the new normal. Clinical trials of AR therapies are associated with high placebo response rates to the degree that the US Food and Drug Administration has indicated AR as one of the few conditions in which including a placebo arm is not only ethically justified but also mandatory.¹ As such, self-reported symptoms used by clinicians to guide therapy are likely often flawed. In most outpatient settings, physicians rely simply on the patient’s history before administering trials of therapy. Potentially, this can lead to unnecessary implementation of additional treatments with potential side effects.

Metabolomics is the study of metabolic pathways and the unique biochemical molecules created in a living system.^{2,3} By measuring changes in metabolite concentrations, the range of biochemical effects induced by a disease or its therapeutic intervention can be determined. Previously, we demonstrated that metabolomic profiling of urine can be used to differentiate healthy children from those with asthma,⁴ children with unstable asthma in the emergency department from those with stable asthma in an outpatient clinic,⁴ and adults with asthma versus those with chronic obstructive pulmonary disease (COPD).⁵

We hypothesized that the metabolic activity of adults with AR would change in accordance with increased nasal airway dysfunction after allergen challenge. We recruited participants with proved ragweed-induced AR and similar age, sex, and body

mass index for ragweed challenge (see Table E1 in this article’s Online Repository at www.jacionline.org; for complete methodology, see the Methods section in this article’s Online Repository at www.jacionline.org). Participants scored their symptoms before and after allergen challenge by using the accepted Total Nasal Symptom Score (TNSS). The TNSS is the sum of 4 individual symptom scores for rhinorrhea, nasal congestion, nasal itching, and sneezing, resulting in a possible score of 0 to 12 (see Table E2 in this article’s Online Repository at www.jacionline.org).

A urine sample was collected from each participant before and after challenge and stored in a -80°C freezer. Metabolites were measured and analyzed for differences between participants’ values before and after allergen challenge in relation to TNSSs.

Initially, we compared the metabolites of one cohort of participants before and after ragweed challenge. We saw that the TNSS severity of each participant was not uniform, even though they were all obtained before allergen challenge (TNSS, 0-5/12). In addition, the reaction to the challenge was not uniform (TNSS, 6-12/12). Based on this, we hypothesized that the metabolome of these subjects also might not be as uniform. To better define a clear metabolomic difference, we needed to better define those who were quite well versus those who had experienced a clear significant allergen challenge response. The TNSS data were used to sort the subject’s prechallenge and postchallenge values. We created a metabolomic model using the subjects with the lowest (0) versus the highest (12) TNSS.

Partial least-squares discriminant analysis (PLS-DA) was performed (SIMCA-P 11; Umetrics, Umea, Sweden). This process identifies metabolites with concentrations that differed significantly between groups of participants expressed as a coefficient of variation plot. Metabolites with consistently greater differences in concentration between groups are displayed by the software as variables of importance to the projection plot (VIP). To choose the most accurate list of metabolites, we removed metabolites listed as being lower in significance on the VIP until we were satisfied that the model could correctly diagnose blinded samples not part of the model. We showed the model the blinded values of metabolites from the remaining participants in group 1 with TNSSs from 1 to 11. The PLS-DA-based model generates a prediction score (0-1) of unclassified blinded data not part of the model. The model blindly assigned scores to these participants (ie, scores >0.5 would be predicted to be TNSS 0, whereas scores <0.5 were predicted to be TNSS 12). We then removed metabolites from the model based on correct blinded analysis scores (scores, 1-11). Using this approach, we created a robust metabolomic model.

The final list of metabolites used to separate mild AR scores from severe scores used 2 components consisting of 11 metabolites, resulting in an R^2 value of 0.85 and Q^2 value of 0.77 (Fig 1, A). Differences in concentrations of these metabolites before and after challenge are shown (Fig 1, B). The final metabolites chosen and their concentrations are shown in Table E3 in this article’s Online Repository at www.jacionline.org.

Graphic presentation of the separation between groups in the model (TNSS, 0 or 12) or test subjects with intermediate TNSSs (1-11) are shown by their respective PLS-DA scores (Fig 1, C). There is a stepwise change in prediction score as the severity of the nasal symptoms increase.

METHODS

Viruses and mice

RSV strain 12/12-6 was isolated in 2012 from a hospitalized infant with severe lower respiratory tract infection and bronchiolitis as part of the Infant Susceptibility to Pulmonary Infections and Asthma Following RSV Exposure (INSPIRE) study.^{E1} RSV was propagated and titrated in HEP-2 cells, as previously described.^{E2} Mock inoculum was prepared by collecting cell-culture supernatants from lysed uninfected HEP-2 cells. Eight-week-old female BALB/cJ mice were purchased from Jackson Laboratories (Bar Harbor, Me). *Il33^{Citrine/+}* reporter mice were generated by crossbreeding WT BALB/c mice and *Il33^{Citrine/Citrine}* mice, which were a kind gift of Dr Andrew N. J. McKenzie.^{E3} Mice were maintained under specific pathogen-free conditions and used in compliance with the revised 2011 “Guide for the care and use of laboratory animals” prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.^{E4} For infection, mice were anesthetized by means of intraperitoneal injection of ketamine/xylazine solution and inoculated through intranasal delivery with 9×10^5 PFU of RSV 12/12-6 or an equal volume of mock inoculum, as previously described.^{E5} Weight loss was measured daily.

The GLP-1R agonist liraglutide (Novo Nordisk, Plainsboro, NJ) was initiated at the same dose used in patients with type II diabetes. This was increased in 2-fold increments daily until the final dose of 0.2 mg/kg weight was reached. The vehicle for liraglutide, 0.1% BSA in PBS, was used as a control. Treatment was administered subcutaneously beginning 2 days before infection or beginning on the same day as infection and given twice daily until the mice were killed.

BAL and periodic acid–Schiff staining

Saline (0.8 mL) was instilled through a tracheostomy tube and withdrawn with a syringe to obtain BAL fluid. Total cells were counted on a hemocytometer by using trypan blue exclusion. For cell differentials, 0.1 mL of BAL fluid was prepared by using a cytospin (Thermo Fisher Scientific, Waltham, Mass) and subsequently fixed and stained with DiffQuik (American Scientific Products, Columbus, Ohio). Differential counts were based on counts of 200 cells using standard morphologic criteria to visualize neutrophils, eosinophils, lymphocytes, or macrophages. For periodic acid–Schiff (PAS) staining, lungs were perfused with PBS, inflated with 10% neutral buffered formalin, and fixed in 10% neutral buffered formalin for 24 hours at room temperature. Lungs were then paraffin embedded, sectioned (5 μ m), and stained with PAS to visualize mucus. Small- and medium-sized airways were scored for mucus by a trained pathologist blinded to experimental information by using the following scoring scheme: 0, no PAS⁺ cells observed in cross-sections of medium to small airways; 1, less than 10 PAS⁺ cells observed in cross-sections of medium to small airways; 2, greater than 10 PAS⁺ cells observed in cross-sections of medium to small airways; or 3, greater than 10 PAS⁺ cells observed in cross-sections of medium to small airways with mucous strands observed in air spaces.

Airway responsiveness

Airway responsiveness was measured, as previously described.^{E6,E7} Briefly, mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (85 mg/kg). A tracheostomy tube was inserted for ventilation. The internal jugular vein was cannulated for intravenous delivery of acetyl- β -methacholine. Mice were then placed in a whole-body plethysmography chamber and ventilated mechanically. Precision glass microsyringes were used to deliver increasing doses of acetyl- β -methacholine. Baseline airway resistance measurements were collected, followed by measurements with 137, 411, 1233, and 3700 μ g/kg body weight of acetyl- β -methacholine (Sigma-Aldrich, Saint Louis, Mo). Peak airway resistance measurements for each dose were recorded.

ELISA

Lungs were snap-frozen in liquid nitrogen at the time of harvest. Lungs were mechanically disrupted with 1 mL of MEM media and homogenized by

using a BeadBeater (BioSpec Products, Bartlesville, Okla). Protein measurements were performed with either DuoSet (IL-33), Quantikine (IL-13, IFN- γ , and IL-27), or Verikine (IFN- α and IFN- β) ELISA kits, according to the manufacturer's instructions (R&D Systems, Minneapolis, Minn). Serum RSV F-protein-specific antibody levels were measured, as previously described.^{E2}

Flow cytometry

Lungs were harvested, minced, and digested in RPMI media with 5% FBS, 1 mg/mL collagenase, and 0.02 mg/mL DNase I for 40 minutes at 37°C. The digestion was stopped with 100 μ L of 0.5 mol/L EDTA, and a single-cell suspension was generated by straining these digestions through a 70- μ m filter. RBC lysis (BioLegend, San Diego, Calif) was performed, according to the manufacturer's instructions. Cells were stimulated in Iscove modified Dulbecco medium media with 10% FBS, 0.01 mmol/L nonessential amino acids, penicillin/streptomycin, 1 mmol/L sodium pyruvate, 10 ng/mL phorbol 12-myristate 13-acetate (PMA), 1 μ mol/L ionomycin, and 0.07% monensin for 4 hours at 37°C. Cells were stained with Live/Dead Blue (Life Technologies, Carlsbad, Calif) and combinations of the following surface markers: CD45 (30-F11), CD25 (PC61.5), Fc ϵ R1 (MAR-1), DX5 (DX5), Fc ϵ R1 (MAR-1), and NKG2D (CX5) from eBioscience (San Diego, Calif); CD127 (SB/199), CD3 (17A2), CD146 (ME-9F1), and epithelial cell adhesion molecule (EpCAM; G8.8) from BioLegend; CD4 (H129.19) from BD Biosciences (San Jose, Calif); and/or a surface marker cocktail containing CD5, CD45R (B220), CD11b, Gr-1 (Ly-6G/C), 7-4, and Ter-119 from Miltenyi (Bergisch Gladbach, Germany). Cells were fixed/permeabilized for 12 hours in Cytofix/Cytoperm (BD Biosciences) and stained with combinations of the following intracellular markers: IL-13 (eBio13A) and IFN- γ (XMGI.2) from eBioscience. Anti-FcR antibody (BD Biosciences) was used to prevent nonspecific staining. Twelve hours of Cytofix/Cytoperm incubation was performed consistently with our laboratory's previous methods.^{E8} We are not concerned with potential epitope cross-linking, which can result in overfixing with a longer fixation time, given that our fluorescence minus one controls uniformly demonstrate a lack of staining. All samples were run on a BD LSR II Flow Cytometer and analyzed with FlowJo software (Version 10; TreeStar, Ashland, Ore).

ILCs were defined as Lin[−]CD45⁺CD25⁺CD127⁺ cells where Lin includes CD3, CD5, CD45R (B220), CD11b, Gr-1 (Ly-6G/C), 7-4, and Ter-119. ILC2s were defined as ILCs that expressed IL-13. T_H cells were defined as CD3⁺CD4⁺ cells, basophils were defined as DX5⁺Fc ϵ R1⁺ cells, NK cells were defined as CD3[−]DX5⁺ cells, and epithelial cells were defined as CD45[−]CD146⁺EpCAM⁺ cells. MFI was determined as the geometric mean.

Viral load

Lungs were snap-frozen in liquid nitrogen at the time of harvest. Thawed lungs were resuspended in 1 mL of sterile MEM media or TRIzol reagent (Invitrogen, Carlsbad, Calif) and homogenized with a BeadBeater (BioSpec Products). Total RNA was isolated by using TRIzol reagent, and cDNA was generated for quantitative PCR analysis of RSV-M and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Commercially available primers and probes for GAPDH were used (catalog no, 4331182, assay ID Mm99999915_g1; Applied Biosystems, Foster City, Calif). Custom primers were designed to measure RSV-M.^{E9} Gene expression was normalized to GAPDH before the fold change was calculated. The fold change in gene expression was calculated through comparison of gene expression with that of lungs from RSV-infected and vehicle-treated mice. Primer sequences were as follows: RSV-M forward, 5'-GGCAAATATGGAAACATACGTGAA-3'; RSV-M reverse, 5'-TCTTTTCTAGGACATTGTAYTGAACAG-3'.

Antibody ELISA

Ectodomain F protein from RSV A2 was fused to a GCN4 trimerization domain and a His tag and expressed in mammalian cells, as previously described.^{E10} Immulon 2B (Thermo Scientific, Rochester, NY) plates were coated with 150 ng of RSV F-protein in PBS overnight at 4°C. Plates were

blocked with 1% BSA in PBS for 1 hour at room temperature. Supernatants were serially diluted 1:2 starting at 1:80 over 6 total dilutions, and plates were incubated for 1 hour at room temperature. RSV-specific antibody was detected by using horseradish peroxidase–conjugated goat anti-mouse antibody specific for mouse IgG (1:5,000), IgG₁ (1:500), or IgG_{2a} (1:500) for 1 hour at room temperature (SouthernBiotech, Birmingham, Ala). Plates were developed in Ultra-TMB (Pierce, Rockford, Ill), and the reaction was stopped with 1 mol/L HCl. Absorbance values (450 nm) were measured and assessed by using Gen5 software (BioTek, Winooski, Vt). The serum end point dilution at 0.2 absorbance units above background (PBS blank) was calculated for each antibody type.

Statistical analysis

Groups were compared by using the unpaired *t* test, 1-way ANOVA with the Bonferroni posttest, or 2-way ANOVA with the Dunn multiple comparison test, as appropriate, with GraphPad Prism software (Version 5; GraphPad Software, San Diego, Calif). Measurements of less than the limit of detection were assigned half of the value of the limit of detection for statistical comparisons.

PheWAS study

To investigate possible human associations with *THADA* variation, we used a population of 29,713 subjects of European ancestry who underwent genotyping on Illumina HumanExome BeadChip version 1.1 and available EMR data from the Vanderbilt BioVU DNA biobank.^{E11} This platform contained the *THADA* SNP rs7578597, which corresponds to a threonine to alanine change in multiple splice variants. The minor T allele of rs7578597 was present in 11% of subjects. We then evaluated all phenotypes defined based on a PheWAS of this SNP by using previously described methods.^{E12} Briefly, the method defines cases for 1000 phenotypes by the presence of specific International Classification of Diseases, Ninth Revision (ICD-9), codes on at least 2 different days. Control subjects for each phenotype are defined as subjects who lack case ICD-9 codes and other codes that are related. For example, cases of the “acute bronchitis and bronchiolitis” phenotype are defined with the ICD-9 code 483, whereas its controls are defined as absence of the ICD-9 code 483. We used version 1.2 of the PheWAS code terminology system and the R PheWAS package to calculate the PheWAS and graph results,^{E13} both of which can be downloaded from <http://phewascatalog.org>. We used logistic regression for each phenotype with 40 cases or more adjusted for age and sex, assuming an additive genetic model.

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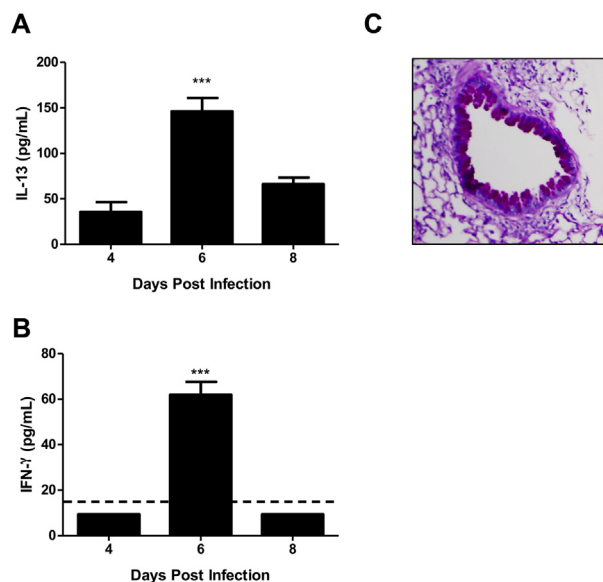


FIG E1. RSV 12/12-6 induces lung IL-13 and mucus production. BALB/cJ mice were infected with 9×10^5 PFU of RSV strain 12/12-6. **A** and **B**, ELISA for IL-13 (Fig E1, *A*) and IFN- γ (Fig E1, *B*) in the whole-lung homogenate (both lungs) 6 days after infection. **C**, Representative PAS-stained section of mucus-containing airway in the lungs 8 days after infection. Data were plotted as means + SEMs. $N = 5$ mice per group. *** $P < .001$, 1-way ANOVA. *Dashed line* is the limit of detection of the assay.

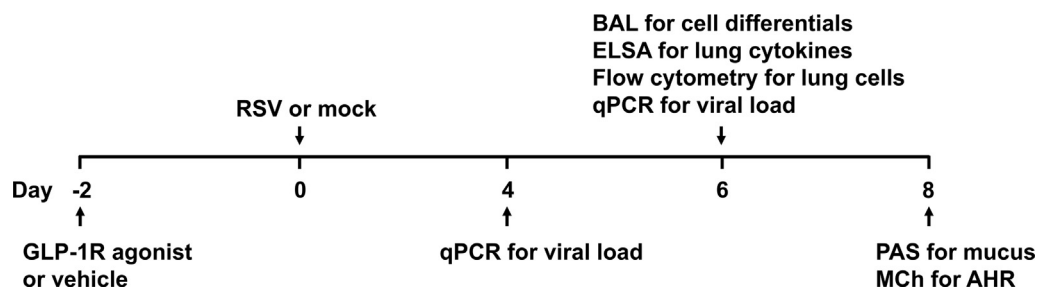


FIG E2. Protocol for *in vivo* administration of GLP-1R agonist or vehicle and subsequent infection with RSV or mock preparation. Treatment with the GLP-1R agonist liraglutide or vehicle (0.1% BSA in PBS) was initiated in BALB/cJ mice on day -2. Mice were infected with 9×10^5 PFU of RSV strain 12/12-6 or mock inoculum on day 0. Treatment was given twice daily until the mice were killed. *AHR*, Airway hyperresponsiveness; *MCh*, methacholine; *qPCR*, quantitative PCR.

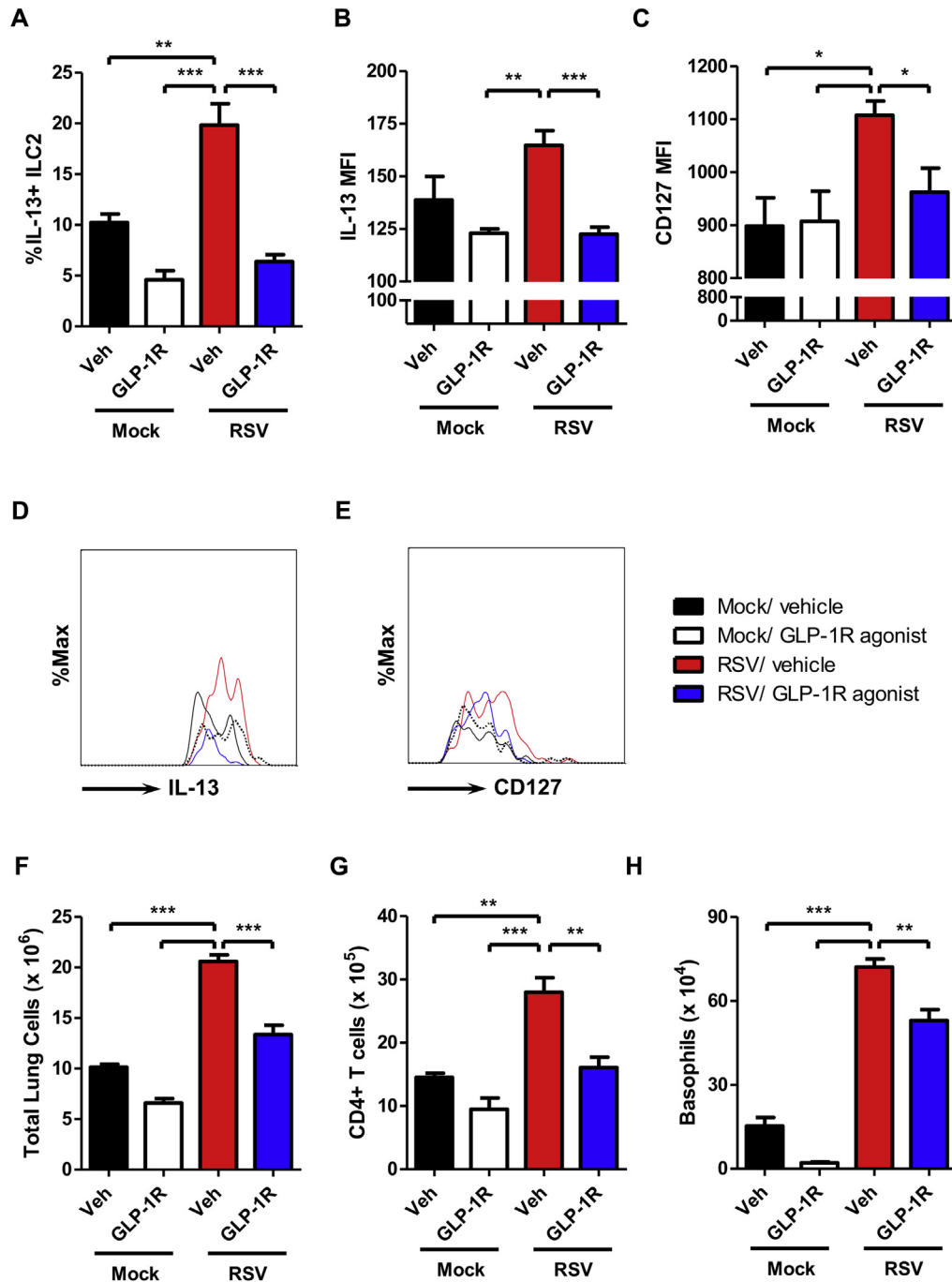


FIG E3. GLP-1R agonist decreases IL-13-producing ILC2s, T_H2 cells, and basophils 6 days after RSV infection. BALB/cJ mice were treated with GLP-1R agonist or vehicle and infected with 9×10^5 PFU of RSV strain 12/12-6 or mock inoculum. **A**, Percentage of ILCs that are IL-13 $^+$. **B** and **C**, MFI of IL-13 (Fig E3, B) and CD127 (Fig E3, C) staining in ILC2s. **D** and **E**, Representative MFI of IL-13 (Fig E3, D) and CD127 (Fig E3, E) staining in ILC2s by using flow cytometry. **F-H**, Total number of live lung cells (Fig E3, F), CD4 $^+$ T_H2 cells (Fig E3, G), and basophils (Fig E3, H). Data are plotted as means $\pm$ SEMs. $N = 3-6$ mice per group representative of 2 independent experiments. * $P < .05$, ** $P < .01$, and *** $P < .001$, 1-way ANOVA.

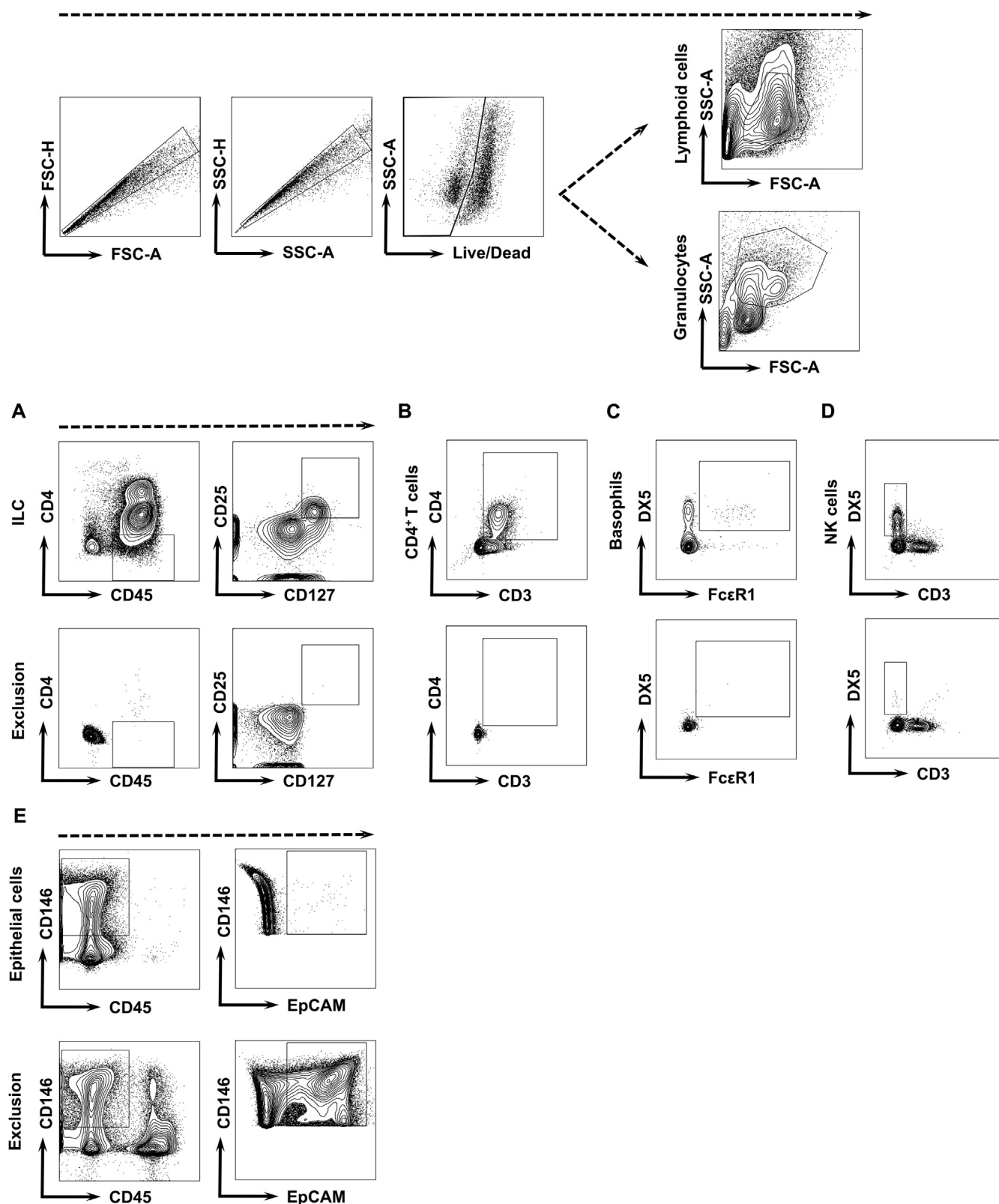


FIG E4. Flow gating for ILCs, CD4⁺ T cells, NK cells, basophils, and epithelial cells. **A**, ILCs were defined as viable Lin⁻CD45⁺CD25⁺CD127⁺ cells. **B**, CD4⁺ T cells were defined as viable CD3⁺CD4⁺ cells. **C**, Basophils were defined as viable FcεR1⁺DX5⁺ cells. **D**, NK cells were defined as viable DX5⁺CD3⁻ cells. **E**, Epithelial cells were defined as viable CD45⁻CD146⁺EpCAM⁺ cells. *FSC*, Forward scatter; *SSC*, side scatter.

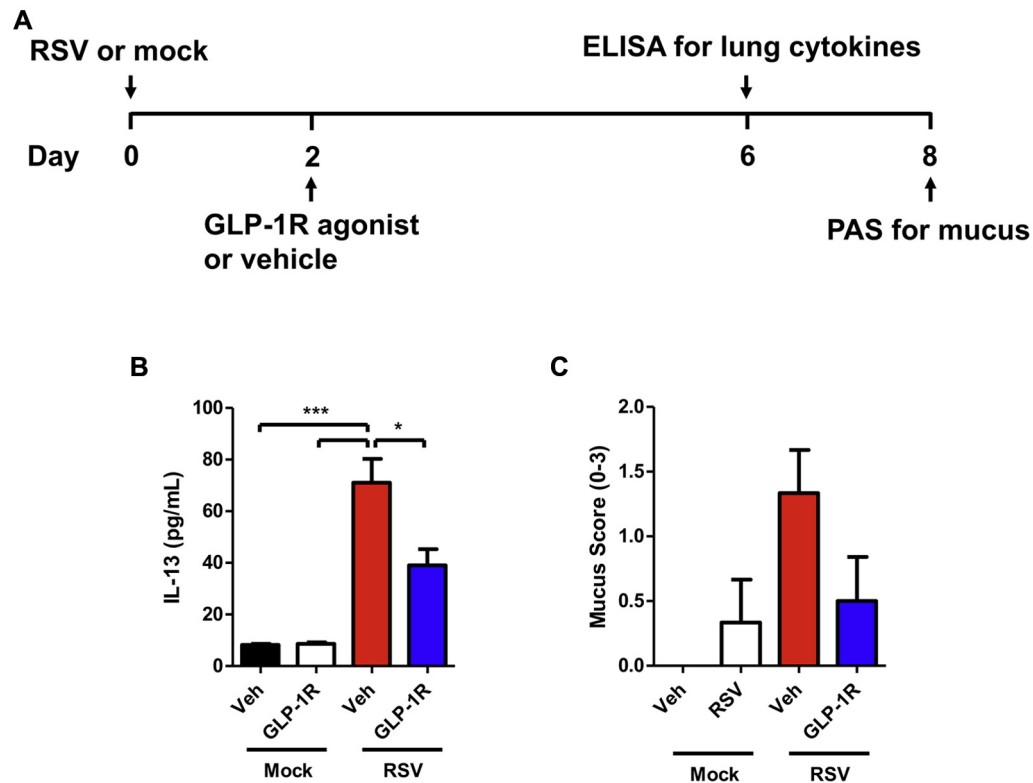


FIG E5. GLP-1R agonist decreases RSV-induced whole-lung IL-13 accumulation and airway mucus production. BALB/cJ mice were treated with GLP-1R agonist or vehicle and infected with 9×10^5 PFU of RSV strain 12/12-6 or mock inoculum. **A**, Protocol for *in vivo* administration of GLP-1R agonist or vehicle and simultaneous infection with RSV or mock preparation. **B**, ELISA for IL-13 in whole-lung homogenate 6 days after infection. **C**, Quantification of airway mucus in the lungs 8 days after infection. Data are plotted as means + SEMs. N = 5 mice per group. * $P < .05$, unpaired t test.

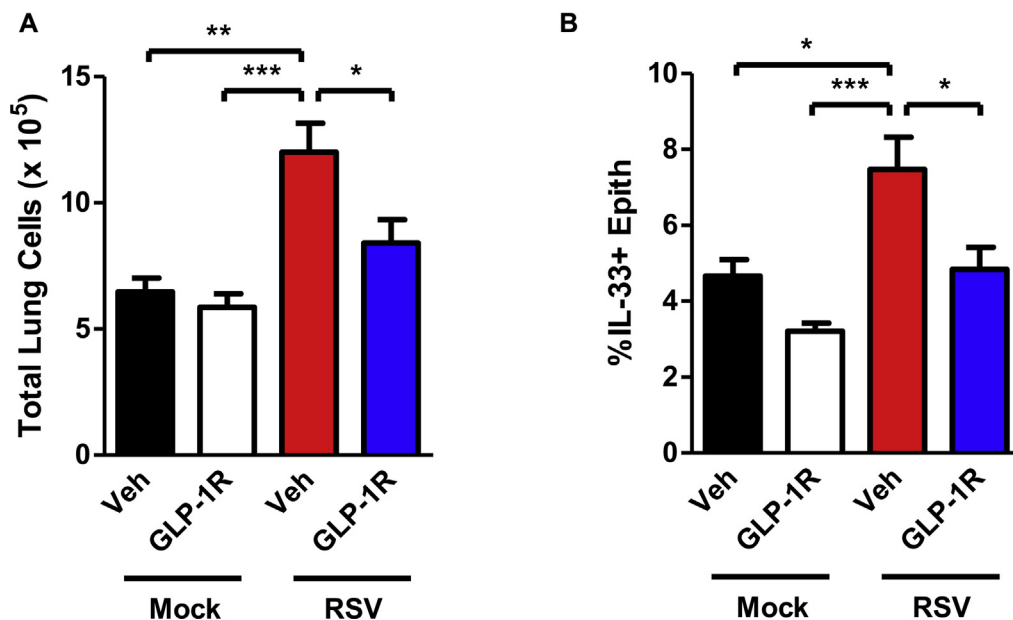


FIG E6. GLP-1R agonist decreases whole-lung IL-33 protein expression and numbers of IL-33-expressing epithelial cells 12 hours after RSV infection. *Il33^{Citrine/+}* reporter mice were treated with GLP-1R agonist or vehicle and infected with 9×10^5 PFU of RSV strain 12/12-6 or mock inoculum. Total numbers of live lung cells (**A**) and percentages of IL-33⁺ epithelial cells (**B**). Data are plotted as means + SEMs. N = 3-6 mice per group representative of 2 independent experiments. **P* < .05, ***P* < .01, and ****P* < .001, 1-way ANOVA.

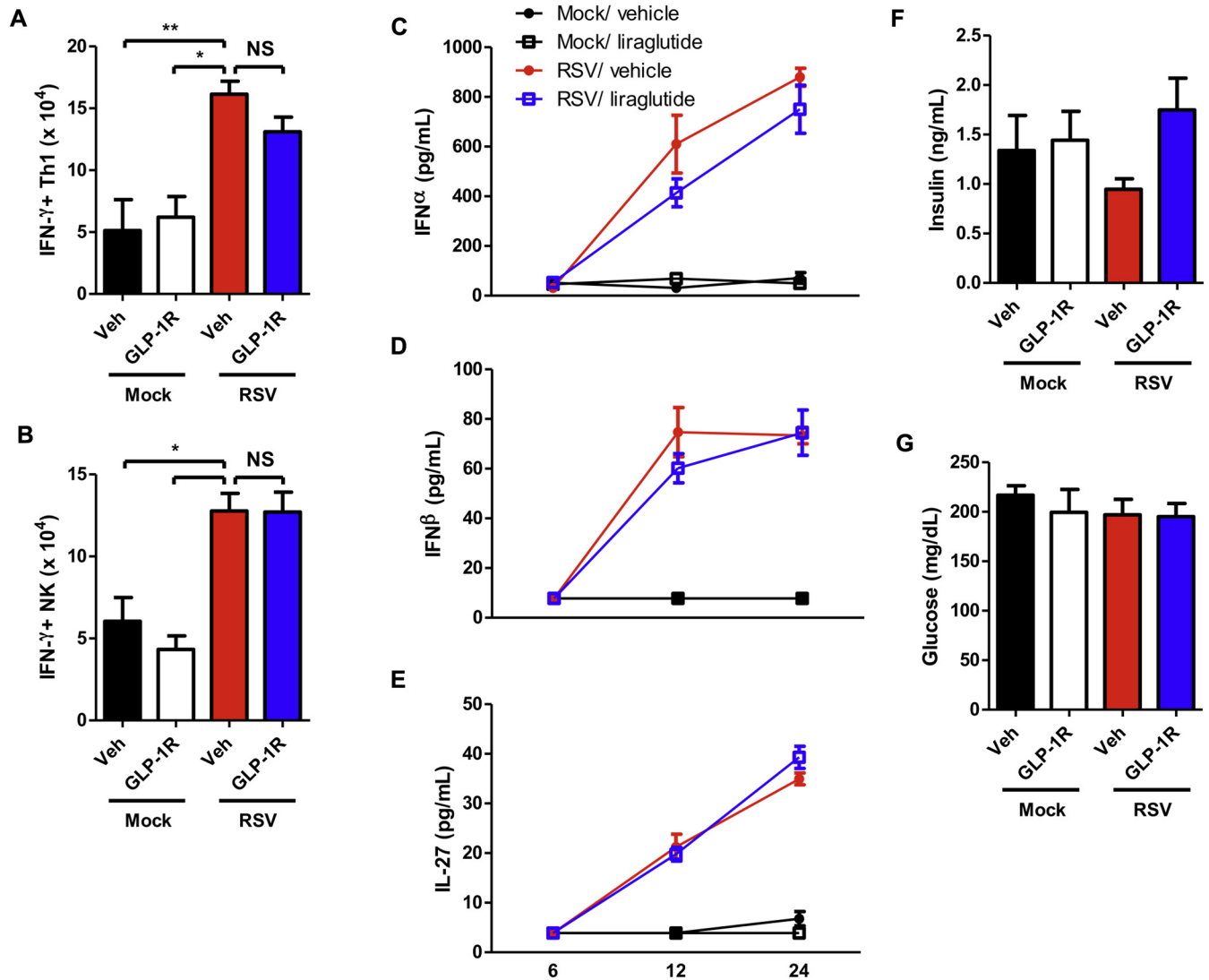


FIG E7. GLP-1R agonist does not decrease interferon responses during RSV infection. BALB/cJ mice were treated with GLP-1R agonist or vehicle and infected with 9×10^5 PFU of RSV strain 12/12-6 or mock inoculum. **A** and **B**, Total number of IFN- γ + Th1 (Fig E7, **A**) and IFN- γ + NK (Fig E7, **B**) cells 6 days after infection. **C-E**, ELISAs for IFN- α (Fig E7, **C**), IFN- β (Fig E7, **D**), and IL-27 (Fig E7, **E**) in whole-lung homogenate 12 hours after infection. **F** and **G**, Serum insulin (Fig E7, **F**) and glucose (Fig E7, **G**) 6 days after infection. Data are plotted as means + SEMs. $N = 3-6$ mice per group representative of 3 experiments (Fig E7, **A-E**) or 1 experiment (Fig E7, **F** and **G**). * $P < .05$ and ** $P < .01$, 1-way (Fig E7, **A**, **B**, **F**, and **G**) or 2-way (Fig E7, **C-E**) ANOVA. NS, Not significant.



FIG E8. Protocol for administration of GLP-1R agonist or vehicle, primary RSV or mock infection, and subsequent secondary RSV or mock infection. BALB/cJ mice were treated with GLP-1R agonist or vehicle beginning on day -2 and infected with 9×10^5 PFU of RSV strain 12/12-6 or mock inoculum on day 0. Treatment was given twice daily until day 8. On day 30, mice were infected a second time with 9×10^5 PFU of RSV strain 12/12-6 or mock inoculum. Abs, Antibodies.

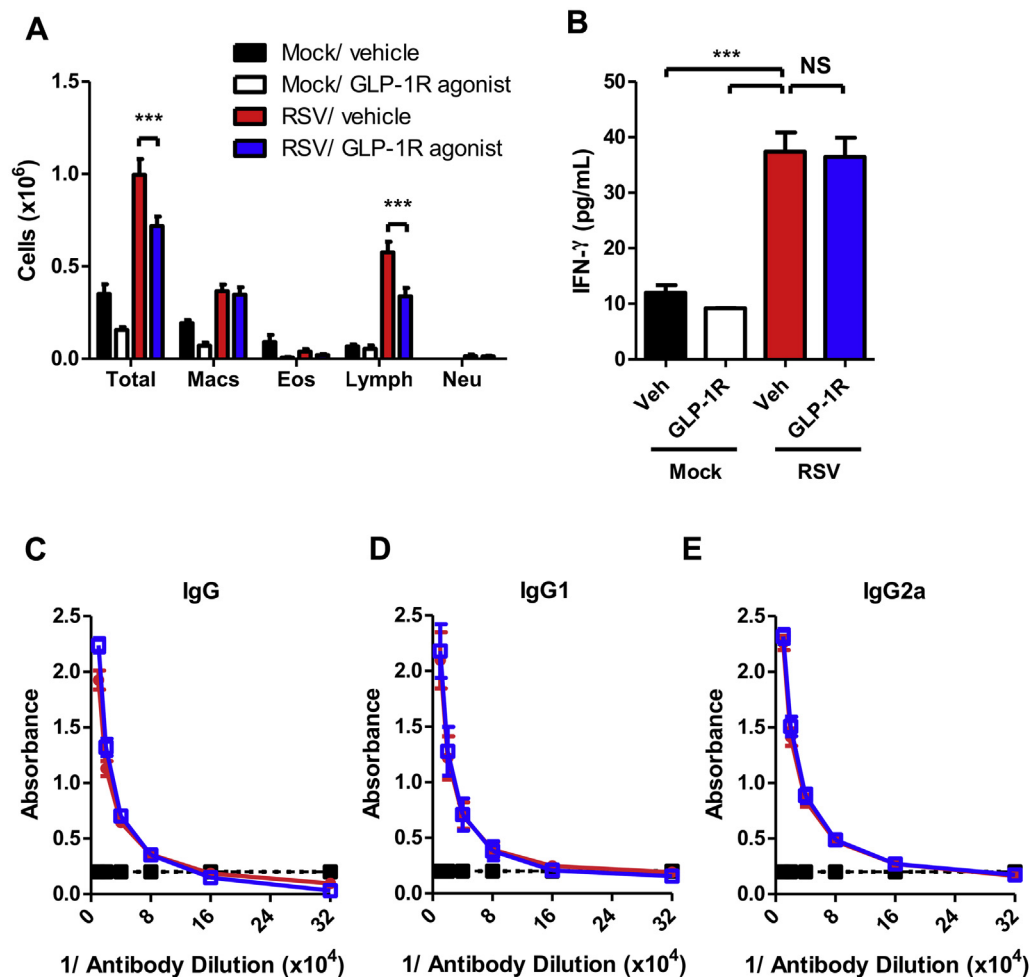


FIG E9. GLP-1R agonist treatment during primary infection prevents airway inflammation and does not reduce anti-RSV antibody responses or lung IFN-γ protein expression during secondary RSV infection. BALB/cJ mice were treated with GLP-1R agonist or vehicle and infected with 9×10^5 PFU of RSV strain 12/12-6 or mock inoculum. Mice were reinfected 30 days after primary infection, and serum was collected 6 days after secondary infection. **A**, BAL fluid cell counts 6 days after infection. **B**, ELISA for IFN-γ in whole-lung homogenate (right lung only). **C-E**, ELISA for RSV F-protein-specific IgG (Fig E8, **C**), IgG₁ (Fig E8, **D**), and (E) IgG_{2a} (Fig E8, **E**). Data are plotted as means + SEMs. N = 6-12 mice per group combined from 2 independent experiments. One-way ANOVA. NS, Not significant.

TABLE E1. PheWAS results for *THADA* rs7578597 in human subjects

Phenotype	Cases	Control subjects	Odds ratio	P value
Scleritis and episcleritis	67	25,573	2.33	.00006
Alkalosis	187	18,873	1.67	.00032
Acute pharyngitis	798	22,135	1.31	.00051
Acute bronchitis and bronchiolitis	1,223	22,347	1.24	.00063
Postinflammatory pulmonary fibrosis	349	19,926	1.43	.00094
Diabetes mellitus	5,032	21,287	0.88	.00136
Type 1 diabetes	468	21,287	0.67	.00157
Morbid obesity	972	24,650	1.23	.00276
Other forms of chronic heart disease	1,095	21,791	1.22	.00359
Other disorders of male genital organs	243	9,538	0.58	.00374
Personality disorders	107	20,065	0.41	.00566
Other symptoms/disorders of the urinary system	4,228	21,638	1.11	.00600
Anxiety, phobic, and dissociative disorders	3,229	20,065	0.89	.00884

All phenotypes with *P* values of less than .01 are reported below.