E-cigarette Use Alters Nasal Mucosal Immune Response to Live-Attenuated Influenza Virus (LAIV)

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ONLINE DATA SUPPLEMENT

Supplementary Materials

Nasal Lavage Fluid (NLF)

Nasal Lavage Fluid (NLF) was collected and processed as described previously[1-7]. Briefly, nasal lavage was collected by irrigating the nasal passages with normal saline (4mL per nostril) and gently expelling the saline and mucus into a sample cup. Nasal lavage was then filtered through a 40μm cell strainer and spun down to separate the cellular components from the cell-free fluid. The cell-free fluid was stored at minus -80°C until analysis and the cells were suspended in Ambion lysis buffer (Life Technologies, Carlsbad, CA) for later RNA extraction and then stored at -80°C.

Nasal Biopsies

Nasal Biopsies (NB) were collected by superficial curettage, scraping the epithelium from the inferior surface of the middle nasal turbinate as in our previous studies[1, 3, 8]. Total RNA was isolated from NB samples using a Pure Link RNA Mini Kit (Life Technologies, Carlsbad, CA). NB RNA was then analyzed for gene expression via Nanostring nCounter PanCancer Immunology code set, with an 8-gene nCounter Panel-Plus add-in to include influenza genes from the 2015-2016 and 2016-2017 seasons of the LAIV FluMist (Table 2). Nanostring data were normalized based on manufacturer instructions.

Nasal Epithelial Lining Fluid (NELF)

NELF was collected on Leukosorb strips (Pall Scientific, Port Washington, NY) and processed as in our previous studies[6]. Leukosorb strips were cut to fit in the nasal passage using a custom metal die and a table top die cutter (Ellison, Lake Forest, CA).

NELF was analyzed using the V-PLEX Human Cytokine 30-Plex Kit from Meso Scale Diagnostics. IP-10 and IL-6 analysis was completed using NLF on single-plex ELISA (BD) and read using a CLARIOstar microplate reader (BMG Labtech Inc.).

Influenza specific subunit replication in NLF cells

RNA was extracted from NLF cells using a Pure Link RNA Mini Kit (Life Technologies, Carlsbad, CA). Messenger RNA was then converted to cDNA and analyzed for Influenza specific subunits using qPCR as described previously[6] using primer/probe pairs specific for the M1 gene of the LAIV Influenza B Ann Arbor/1/66 master donor strain: 5'-FAM-CCCTCTTGTTGTTGCCGC-TAMRA-3' (probe), 5'-GGGTGCAGATGCAACGATT-3' (sense), and 5'-AATATCAAGTGCAAGATCCCAATG-3' (antisense). Data were normalized using *β-actin* mRNA expression and expression differences were evaluated using the ΔCt method.

NLF Virus-specific IgA

Levels of influenza-specific IgA in the NLF were measured using a direct sandwich ELISA as described and validated in the literature[9, 10]. High binding, half-area ELISA plates (Corning, 3690) were coated overnight at 4°C with 50µL of 50 HAU/mL control IAV antigen (Influenza Reagent Resource, A/California/7/2009, FR-1184) corresponding to the strain present in the nasal vaccine diluted in ELISA coating buffer (BioLegend, 421701). Standard curves were generated by coating wells overnight with serial dilutions of human IgA (Invitrogen, 31148) and control wells for each sample were coated with 0.5% BSA in coating buffer. Plates were blocked with 1% BSA in PBS for 1 hour. Prior to addition to the plate, NLF samples were incubated 1:1 with 10mM DTT at

37°C for 1 hour and then diluted in 1% BSA in PBS to a final dilution of 1:6. Samples were incubated on the plate overnight at 4°C. Plates were washed and then probed with a goat anti human IgA-HRP antibody (Bethyl Labs, A80-102P 1:10000) for 2 hours at room temperature shielded from light. Plates were then washed and developed using TMB solution (BD Biosciences, 555214) and stopped with 2N H_2SO_4. Virus specific IgA levels were determined against the IgA standard curve. The levels of virus specific IgA were then normalized against total IgA levels, which were measured against a commercially available sandwich ELISA per manufacturer instructions (Thermo Fisher Scientific, BMS2096). For reference, Mean ± SEM (ng/mL) for total IgA: 70480 ± 5726, 58364 ± 4977, and 89879 ± 16925 for cigarette smokers, e-cigarette users, and non-smokers, respectively. Mean ± SEM (ng/mL) for virus specific IgA pre-exposure: 320.1 ± 44.39, 344.6 ± 30.94, and 325.5 ± 35.89 for cigarette smokers, e-cigarette users, and non-smokers, respectively. Mean ± SEM (ng/mL) for virus specific IgA post-exposure: 340.3 ± 43.85, 325.5 ± 35.89, and 365.6 ± 31.52 for cigarette smokers, e-cigarette users, and non-smokers, respectively. Normalization was calculated by dividing the virus specific IgA levels by total IgA levels and multiplying by 100 as previously described[11]. The change in antibody levels was determined by dividing the virus-specific antibody concentration post-LAIV from pre-vaccination levels and multiplying by 100 for a relative percentage.

Supplementary Tables:

Supplementary Table E1: Differentially expressed (DE) genes at baseline in cigarette smokers (left) and e-cigarette users (right) as compared to non-smokers **A) Males** and **B) Females**. Genes that are differentially expressed in both exposure groups within the same sex are highlighted in blue at the top of each sub-table. Genes that are differentially expressed in both sexes within the same exposure group are highlighted in grey. Significance was determined by a p-value of less than or equal to 0.05 and a fold change of greater than or equal to the absolute value of 1.5.

Supplementary Table E2: Differentially expressed genes in response to LAIV using baseline subtracted AUC with aggregate data in cigarette smokers (left) and e-cigarette users (right) as compared to non-smokers. Genes that are differentially expressed in both exposure groups are highlighted in blue in the top left of each column. Significance was determined by a p-value of less than or equal to 0.05 and a percent change from non-smoker of greater than or equal to the absolute value of 150 percent.

Supplementary Table E3: Nasal protein response to LAIV. Responses to LAIV in each exposure group as measured by AUC over days 0, 1, 2, 8. P-values are shown for exposure group listed compared to nonsmoker controls. Significance of p less than or equal to 0.1 is denoted by bolded text in a grey cell.

Supplementary Table E4: Interaction of Sex and Tobacco Product Exposure on Response to LAIV. Genes that display an interaction by sex and tobacco product exposure using baseline subtracted AUC are reported to the left of the table with the p-value for interaction. In the right sections of the table, the AUC for non-smokers and

percent change in each group from non-smokers by cigarette use and e-cigarette use are reported by sex. *Significantly different compared to non-smokers within sex stratified analyses. Significance was determined by a p-value of less than or equal to 0.05 and a percent change from non-smoker of greater than or

Supplementary Table E5: Top 25 KEGG pathways enriched in sex*tobacco product exposure interaction network

Supplementary Table E6: MCL clustering description

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