

METHODS

PAFR expression: Stored mouse lung tissue

Mice inhaled StS-WF composed of (weight percentage) iron (57%), chromium (20%), manganese (13%), nickel (8%), and copper (0.2%) with trace amounts of silicon, aluminum, and vanadium. The particle diameters ranged from ultrafine (0.01-0.1 μm) to coarse (1.0-10 μm), with the majority of particles in the fine size range (0.1-1.0 μm). The mass median aerodynamic diameter was 0.255 μm , with a geometric SD of 1.35. RNA from mice exposed to aerosolized StS-WF was isolated from whole-lung homogenates by using TRIzol (Invitrogen) and then cleaned according to the manufacturer's instructions with an RNeasy Mini Kit (Qiagen). A 2 μL aliquot of each RNA sample was quantified by using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Del). Briefly, RT-qPCR reactions were carried out by using StepOne (Applied Biosystems, Foster City, Calif) with predesigned Assays-on-Demand TaqMan probes and primers (Applied Biosystems). By using 96-well plates, 1 μg of total RNA was reverse transcribed with random hexamers (Applied Biosystems) and Superscript III (Invitrogen). Hypoxanthine-guanine phosphoribosyltransferase was used as the reference gene. Relative gene expression was calculated by using the

comparative cycle threshold ($\Delta\Delta C_T$) method. All procedures and protocols were approved by the Animal Care and Use Committee of the National Institute for Occupational Safety and Health.

PAFR expression: Stored human lung tissue

PAFR antigen retrieval in human lung biopsy tissue was carried out on 3- μm paraffin wax-embedded sections dried overnight at 60°C. Slides were placed in an EDTA buffer of pH 8.1 and microwaved at full power for 35 minutes. Slides were then transferred to a DAKO autostainer (DAKO, Glostrup, Denmark), where they were treated with a 3% peroxidase block followed by using the R.T.U Vectastain Kit (PK-7200; Vector Laboratories, Burlingame, Calif), according to the manufacturer's recommendations. The working dilution of the human anti-PAFR mAb CAY160600 (Cayman Chemical) was used at 1:100, and the incubation time was 40 minutes. The signal was visualized by using DAKO DAB+ Chromogen Solution (K3468) applied for 5 minutes. A Gills hematoxylin nuclei counterstain was used for 2 minutes. A negative control using tonsil tissue without the anti-PAFR antibody showed no nonspecific diaminobenzidine signal.

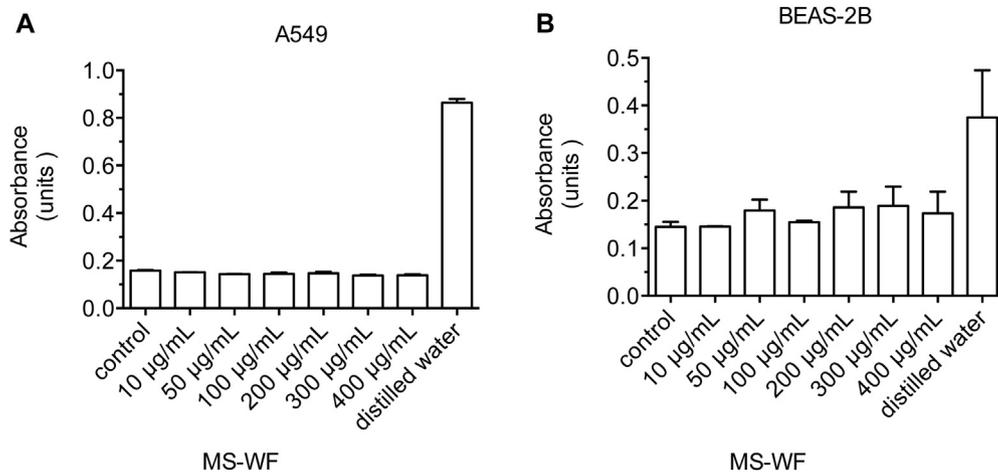


FIG E1. LDH release from A549 cells exposed to 2 hours of MS-WF (**A**) and BEAS-2B cells exposed to 2 hours of MS-WF (**B**). Data are from a single experiment, with 3 technical replicates. The positive control is assessed after total cell lysis by using distilled water. MS-WF at concentrations of 400 $\mu\text{g/mL}$ or less do not cause cytotoxicity in this assay.

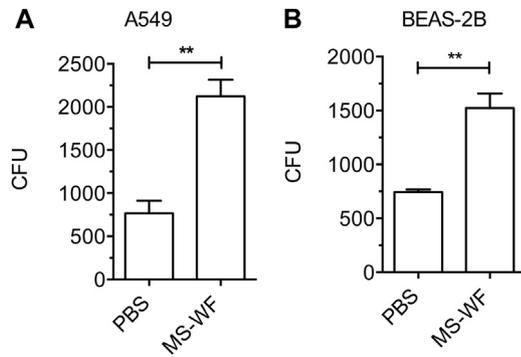


FIG E2. Effect of exposure of airway cells to 10 $\mu\text{g/mL}$ ($5 \mu\text{g/cm}^2$) MS-WF *in vitro* for 24 hours on the adhesion of *S pneumoniae* to A549 cells (**A**) and BEAS-2B cells (**B**). Increased CFU values determined by using quantitative culture reflect increased pneumococcal adherence. Data are from 3 separate experiments, described as means (SEMs), and compared by using the *t* test. ***P* < .01 versus medium control.

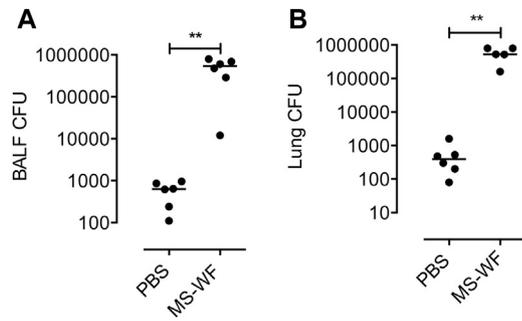


FIG E3. Effect of exposure of mice to 600 μg of intranasal MS-WF administered as 100- μg doses once a day for 6 days on *S pneumoniae* CFU values in BALF (**A**) and lung tissue (**B**). Mice were infected 24 hours after instillation of the last dose of MS-WF, and CFU values were assessed by means of qualitative culture. Data are from 6 animals per group and compared by using the Mann-Whitney *U* test. Bars represent medians. ** $P < .01$.

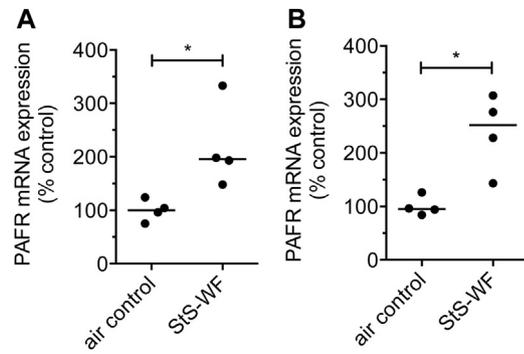


FIG E4. Effect of aerosolized StS-WF on mouse lung PAFR mRNA expression. PAFR mRNA expression was assessed by using real-time quantitative PCR with hypoxanthine-guanine phosphoribosyltransferase as the reference gene. Relative gene expression was calculated by using the $\Delta\Delta$ cycle threshold method. **A**, Four hours after a 10-day course of aerosolized StS-WF (40 mg/m^3) for 3 hours. **B**, Twenty-eight days after a 10-day course of 3 hours per day of aerosolized StS-WF (40 mg/m^3). Dot plots are from 4 mice per group. Data are compared by using the Mann-Whitney test. Bars represent medians. $*P < .05$.

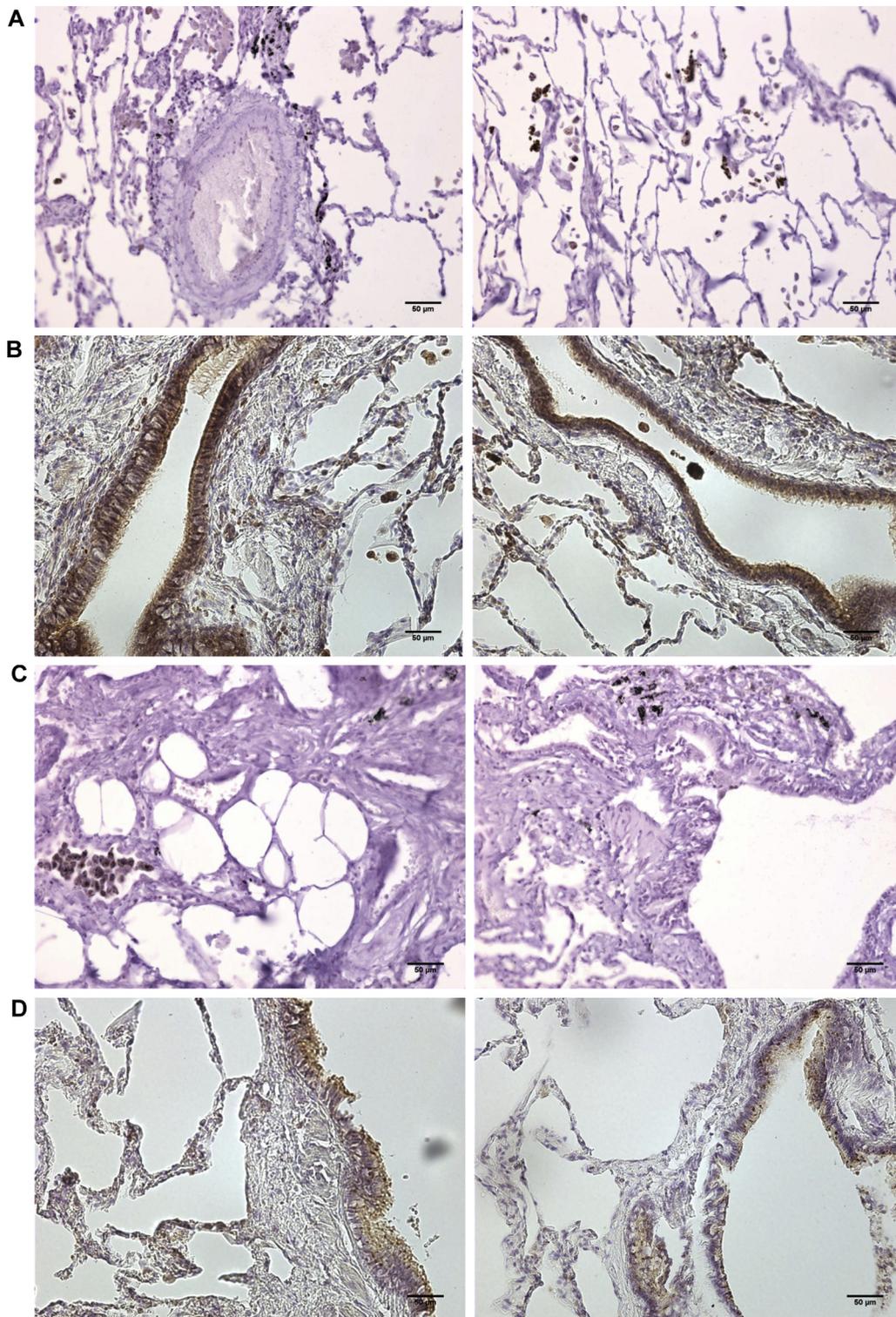


FIG E5. PAFR immunostaining in the human lung. A biopsy specimen of normal tissue was obtained at the time of biopsy for malignancy. **A**, Lung tissue from a nonsmoking welder stained with an isotypic control mAb. There is no specific (*brown*) staining of epithelial cells. **B**, Lung tissue from a nonsmoking welder stained with a PAFR mAb. There is marked specific staining of bronchial epithelial cells and some specific staining of alveolar epithelial cells. **C**, Lung tissue from a nonsmoking, non-WF-exposed control subject stained with an isotypic control mAb. **D**, Lung tissue from a nonsmoking, non-WF-exposed control subject stained with a PAFR mAb. There is specific PAFR staining of bronchial epithelial cells (*brown*).

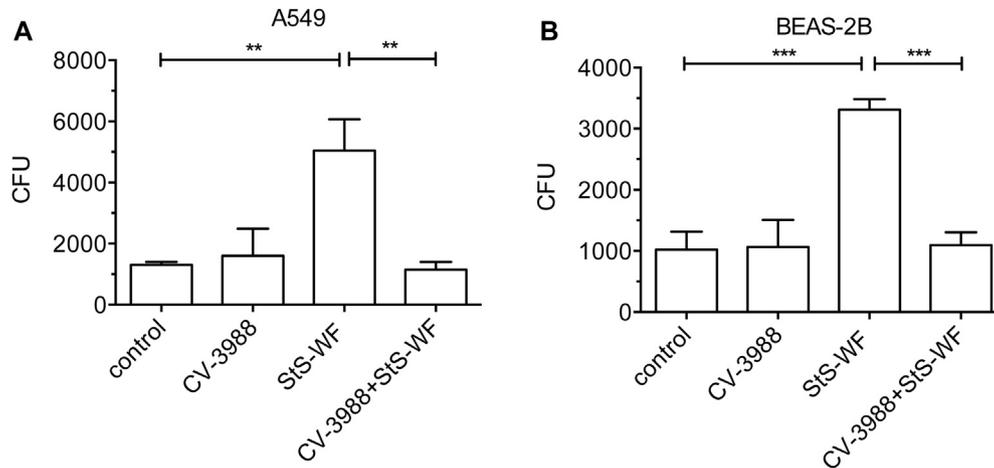


FIG E6. Effect of the PAFR blocker CV-3988 (20 $\mu\text{mol/L}$) on adhesion of *S pneumoniae* to airway cells after 2 hours of exposure to StS-WF. StS-WF (chromium, 4.1%; iron, 3.9%; manganese, 2.7%; and titanium, 1.5%) were generated as described in the Methods section by using E308L manual metal arc welding electrodes. Increased CFU values determined by using quantitative culture reflect increased pneumococcal adhesion and infection. **A**, A549 cells plus 275 $\mu\text{g/mL}$ StS-WF. **B**, BEAS-2B cells plus 200 $\mu\text{g/mL}$ StS-WF. StS-WF stimulates pneumococcal adhesion, and this is attenuated by CV-3988. Data are from 4 separate experiments, with 3 replicates per experiment. Data are described as means (SEMs) and compared by using 1-way ANOVA and the Tukey multiple comparison test. * $P < .05$, ** $P < .01$, and *** $P < .001$.