Measurement of IL-13–Induced iNOS-Derived Gas Phase Nitric Oxide in Human Bronchial Epithelial Cells

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ONLINE DATA SUPPLEMENT

MATERIALS AND METHODS

Materials. IL-13 was purchased from R&D Systems (Minneapolis, MN), iNOS inhibitor N^6-(1-iminoethyl)-L-lysine (L-NIL, IC50 3.3 μM for iNOS, and 92 μM for nNOS) from Cayman Chemicals (Ann Arbor, MI), nNOS inhibitor L-N_ω-nitroarginine-2,4-L-diaminobutyric amide (L-NA-DBA, IC50 55 μM for iNOS, and 1.13 μM for nNOS) from Sigma (St. Louis, MO). Antibodies for iNOS (mouse monoclonal 1E8-B8) and nNOS (rabbit polyclonal) were from Research and Development Antibodies (Las Vega, NV). Griess assay kit for nitrite and nitrate quantification was obtained from Biovision (Mountain View, CA).

Cell Culture. Cryopreserved passage 1 normal human bronchial epithelial (NHBE) cells from 3 different donors (donor 1: specific lot number not known, donor 2: 4F1430, donor 3: 4F1624) were obtained from Cambrex (Walkersville, MD). Cells were thawed and passaged twice in T-75 flasks (Fisher) in a 37° C 5% CO_2/95% air incubator in bronchial epithelial growth medium (BEGM) (Cambrex) supplemented with prescribed growth factors supplied in the SingleQuot Kit (Cambrex). Cells were trypsinized when 70-80% confluent and seeded at passage 3 onto Costar Transwell® inserts (Fisher) at a density of 1.35×10^5 cells/cm^2 using culture medium and supplements described previously (1). Culture medium was changed the day after seeding and 3 times a week after that. The cells were grown submerged till confluent (2-4 days after seeding) and then switched to an air-liquid interface condition for 14 days to achieve mucociliary differentiation.

Reverse Transcription and PCR. Total RNA was isolated and quantified. Reverse transcription was carried out using TaqMan® reagents (Applied Biosystems, Foster City, CA). Gene expression of the NOS isoforms was probed via PCR using the primers 5'-GCCTCGCTCTGGAAAGA-3', 3'-TTCCAACAGACGTACCT-5' (iNOS); 5'-AGGACAGACGGCAAGCACGA-3', 3'-GGTGGCGGAGTGATGGTCAA-5' (nNOS); 5'-CAGTGTCCAACATGCTGCTGGAAATTG-3', 3'-CCGTAGTGGTCCTTCTTCTGGAAAT-5' (eNOS). Ribosomal 18S RNA (Ambion) was used as an internal standard. Products were visualized by ethidium bromide staining.

Western Analysis. Protein was extracted as described previously (2) and quantified using the Bradford assay (BioRad). Protein (20 µg) was resolved by 7.5% SDS-PAGE at 130V, transferred to PVDF membrane (Millipore) (0.4 A, 90 minutes), probed for iNOS and nNOS following the antibody manufacturer's recommendations and visualized using an enhanced chemiluminescence system (Amersham Biosciences). The blots were stripped and reprobed with a monoclonal anti-β-actin antibody to verify equal loading.

REFERENCES

E1. Gray, T. E., K. Guzman, C. W. Davis, L. H. Abdullah, and P. Nettesheim. 1996. Mucociliary differentiation of serially passaged normal human tracheobronchial epithelial cells. *American Journal of Respiratory Cell and Molecular Biology* 14(1):104-112.

E2. Kolodziejski, P. J., A. Musial, J. S. Koo, and N. T. Eissa. 2002. Ubiquitination of inducible nitric oxide synthase is required for its degradation. *Proc Natl Acad Sci U S A* 99(19):12315-20.