SUPPLEMENTARY MATERIAL

Subjects

Nasal mucosa (NM) specimens were obtained from seven subjects (one woman, 34±2.9 yr) who had septal deviations or turbinate hypertrophy and underwent corrective surgery (control-NM). Nasal polyp (NP) specimens were collected from seven patients with AERD (four women, 48.3±2.6 yr) and from seven non-AERD patients (three women, 44.3±16.6 yr) who were treated with endoscopic surgery. The diagnosis of aspirin intolerance was confirmed by utilising an oral or lysine-aspirin nasal challenge. None of the subjects received oral or intranasal corticosteroids for at least one week before the operation or had upper airway infections in the two weeks before the operation. Control and non-AERD subjects had taken aspirin or NSAIDs without adverse reactions (asthma, rhinitis, urticaria, angioedema, or anaphylaxis). The laboratory staff involved in the ELISA study were unaware of which group the samples came from.

All patients provided informed consent to participate in the study, which was approved by the scientific and ethics committee of our institution.

Tissue handling, cell culture, and PGD_2 measurement

NM and NP samples were cut into 33-mm fragments and placed in 6-well plates (NUNC, Wiesbaden, Germany) containing Dulbecco's modified Eagle medium supplemented with 10% FBS, 100 IU/mL penicillin, 100 mg/mL streptomycin (Invitrogen, Carlsbad, Calif), and 2 mg/mL amphotericin B (Sigma, St Louis, Mo). Cultures were kept in a 5% CO_2 humidified incubator at 37ºC. When fibroblasts began to proliferate and covered 50% of the well surface, tissue fragments were removed, and the first passage was performed by adding 0.05% trypsin/0.02% EDTA (Invitrogen) for 5 minutes. The reaction was stopped with 10% FBS-supplemented media, and cells were centrifuged (400 x *g* for 5 minutes), seeded in 75-cm^2 flasks (NUNC), and grown to 80% confluence. At passages 3 to 7, fibroblasts were cultured to subconfluence in CultureSlides and 150 cm^2 flasks (NUNC, Rochester, NY) to perform culture characterisation and experimental protocols. Culture characterisation was performed using immunofluorescence for vimentin (fibroblasts), cytokeratin (epithelial cells), and smooth muscle actin (myofibroblasts) in CultureSlides incubated with SFM for 24 hours. The same batch of FBS was used for the entire experimental period, and mycoplasma contamination was tested using PCR in all cultures; none were found to be positive.

Experimental protocols began when cultures were subconfluent and after incubation with serum-free medium (SFM) for 24 hours. Then, the culture medium was changed, and cells were incubated with SFM in the presence or absence of 10 ng/mL IL-1β (R&D Systems, Minneapolis, Minn) for different time periods.

Culture supernatants were collected, centrifuged at 400 x *g* for 10 minutes at 4ºC, sterilised through 0.22-mm filters, and stored at -80ºC until PGD_2 concentrations were measured. Total protein was obtained by collecting the cells after two washes with ice-cold PBS. The cells were centrifuged (1500 x *g* for 5 minutes at 4ºC) and resuspended in 0.4 mL of ice-cold lysis buffer (Complete protease inhibitor cocktail tablet in 50 mL of 0.05 mol/L Hepes buffer solution, 0.05% vol/vol Triton X-100, and 625 mmol/L phenylmethylsulfonyl fluoride).

doi: 10.18176/jiaci.1062

Cells were sonicated twice for 15 seconds in a sonifier (Branson, Danbury, Conn) and centrifuged (12,000 x *g* for 10 minutes at 4ºC).

Concentrations of PGD_2 in supernatants were measured with enzyme immunoassay kits (Cayman Chemical, Ann Arbor, Mich). The assay range was 7.81 to 1000 pg/mL. Concentrations of PGD_2 were normalised to the total protein content in the cell lysate from corresponding samples.

Statistical analyses

ELISA results are shown as medians (25th-75th interquartile) in picograms of PGD_2 per microgram of total proteins. The nonparametric statistical Mann-Whitney U test was used for between-group comparisons and the Wilcoxon test for paired comparisons. Statistical significance was set at a *p*-value < .05.

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