Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix

1. Collection and processing of tonsillar tissue

After surgical excision, tonsillar tissue was placed in phosphate-buffered saline (PBS) and was transferred rapidly to the Pathology Laboratory for further processing and use in RT-qPCR, flow cytometry and confocal laser scanning microscopy. Part of the tissue sample was fixed in buffered formalin 4%, processed routinely, and embedded in paraffin. Sections were cut at 3 μm using a microtome and dried overnight at 60°C. The remaining tissue was mechanically dissociated in RPMI, was centrifuged and the cell pellet was resuspended in RPMI for use in quantitative real-time polymerase chain reaction (RT-qPCR) and in flow cytometry. For RT-qPCR, tonsillar lymphocytes in the cell suspension were separated in T- and B-lymphocytes using BD IMag anti-human CD3 or CD19 Particles–DM (BD Biosciences; San Jose, CA) and a magnetic field (BD IMagnet, Cat. No. 552311).

2. RT-qPCR

Total RNA was extracted from T- or B-lymphocyte fractions with NucleoSpin RNA/Protein kit (Macherey-Nagel; Düren, Germany) and its concentration and quality were determined using a spectrophotometer (Genova, Jenway; Stone, Staffordshire, UK). 1.0 mcg of total RNA was reverse-transcribed into cDNA by Moloney Murine Leukemia Virus Reverse Transcriptase using oligo(dT) as reverse transcription primer (PrimeScript RT-PCR Kit, TaKaRa Bio Europe; Saint-Germain-en-Laye, France). The reverse transcription reaction mixture was incubated for 50 min at 42°C and the reaction was terminated for 15 min at 70°C.

cDNA equivalent to 5.0 ng of total RNA was subjected to RT-qPCR in triplicates in a Mx3005P RT-qPCR system (Stratagene; La Jolla, CA) according to the manufacturer’s protocol (KAPA SYBR Fast Universal qPCR kit, KAPA Biosystems; Woburn, MA). The cycling conditions consisted of one cycle at 95°C for 3 minutes, 40 three-segment cycles (95°C for 3s, 60°C for 30s and 72°C for 11s) and a final dissociation cycle (95°C for 60s and stepwise increase from 55°C to 95°C). Each of the calculated cycle thresholds for 5-LO, FLAP, LTA4H and LTC4S was normalized against the corresponding b-actin cycle threshold. Thus, 5-LO, FLAP, LTA4H and LTC4S were expressed as ratios of the gene of interest value to the corresponding b-actin value. PCR results were analyzed using the Mx3005P software (Stratagene).

PCR primers for 5-LO, FLAP, LTA4H and LTC4S and b-actin (Invitrogen; Camarillo, CA) were: 5-LO forward primer, 5'-ACTGGAAACACGGCAAAAAC-3'; reverse primer, 5'-TTTCTAAAGTCCGGCGGAAGT-3' (60°C annealing temperature, 226bp product size); FLAP forward primer, 5'-GGCTTTGTCTGGACTGATGTA-3'; reverse primer, 5'-TGGTGGTGGAGATCGTCTTT-3' (60°C annealing temperature, 211bp product size); LTA4H forward primer, 5'-CAGTGGCTCATCGTGAAC-3'; reverse primer, 5'-TCTGGGTAGTGCCTTTCC-3' (60°C annealing temperature, 211bp product size); LTC4S forward primer, 5'-ACACCGACGGTAACCATTGAAG-3'; reverse primer, 5'-GGGAAGTCATCGTGAGTT-3' (60°C annealing temperature, 196bp product size).

3. Confocal laser scanning microscopy

Confocal laser scanning microscopy was used to localize T- and B-lymphocytes expressing leukotriene biosynthetic enzymes within the tonsillar tissue. A double-stain protocol was applied in adjacent formalin-fixed, paraffin-embedded tissue sections. Paraffin sections were deparaffinized by the standard laboratory procedure (xylene, ethanol and distilled H2O washes). The sections were then washed twice in PBS pH 7.4 and incubated for 30 minutes in a steamer with Target Antigen Retrieval (DakoCytonmation; Glostrup, Denmark). Two further washes in PBS were completed followed by incubation of the sections for 10 min in hydrogen peroxide (H2O2).

After three washes with PBS and antigen blocking by protein blocking solution (DakoCytonmation), histologic sections were incubated overnight with primary rabbit polyclonal antibodies against: i) 5-LO (dilution 1:100; Cayman Chemical; Ann Arbor, MI); or ii) FLAP (dilution 1:100; Santa Cruz Biotechnology; Santa Cruz, CA); or iii) LTA4H (dilution 1:100; Cayman Chemical); or iv) LTC4S (dilution 1:100; Sigma-Aldrich; St Louis, MO) at 4°C in a humidifier. The following day, histologic
sections were washed in PBS and incubated for 30 minutes at room temperature with DyLight 488 secondary anti-rabbit antibody (dilution 1:200; Thermo Fisher Scientific UK; Loughborough, UK).

The slides were washed again with PBS and incubated for 1 hour at room temperature with the second primary antibody: i) mouse anti-human CD20 (dilution 1:200; Thermo Fisher Scientific); or ii) rabbit anti-human CD3 (dilution 1:200; Thermo Fisher Scientific). Following further washes with PBS, sections were incubated for 30 minutes with DyLight 549 secondary anti-mouse antibody (dilution 1:200; Thermo Fisher Scientific) for sections with anti-CD20 and AlexaFluor 564 secondary anti-rabbit antibody (dilution 1:200; Life Technologies-Molecular Probes; Grand Island, New York) for sections with anti-CD3. Finally slides were washed with PBS and counterstained with DAPI. Sections were then examined by an Olympus FV1000 Confocal Laser Scanning Microscope using the Olympus FluoView ASW-2.1 software (Olympus; Tokyo, Japan).
**eFigure 1.** Tonsillar lymphocyte subpopulations demonstrated by flow cytometry

Analysis of CD3+ T- and CD19+ B-lymphocyte populations in tonsillar tissue demonstrated by flow cytometry. Representative case of a child with obstructive sleep apnea who underwent adenotonsillectomy.
**eFigure 2.** Leukotriene biosynthetic enzymes expression in tonsillar lymphocyte subpopulations demonstrated by flow cytometry

Flow cytometry analysis of leukotriene biosynthetic enzymes in tonsillar CD3+ T-lymphocytes (left panels) and CD19+ B-lymphocytes (right panels). Representative case of a child with obstructive sleep apnea who underwent adenotonsillectomy.