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Unique and Overlapping IL-4 and IL-13 Gene Expression Patterns Driven in the Mouse Lung

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RNA Isolation and Microarray Hybridization: Lungs were frozen in liquid nitrogen immediately after harvest and homogenized in Trizol Reagent® (GibcoBRL, Carlsbad, CA) using an ULTRA-TURRAX power homogenizer. Total RNA was isolated and purified using a standard protocol (TRIzol Reagent® method) followed by RNAeasy purification (Qiagen, Valencia, CA). 50 μg of cleaned total RNA was processed for double stranded cDNA production using a T7 promoter-tailed oligo-dT primer from SuperScript Choice system (GibcoBRL, Carlsbad, CA). Biotinylated cRNA was produced using the ENZO Bioarray RNA transcripts labeling kit (Qiagen, Valencia, CA), and fragmented randomly to approximately 200 bp (200mM Tris-acetate, pH 8.2, 500 mM KOAc, 150mM MgOAc). Equal amounts of RNA were pooled from each individual mouse lung in an experimental group (n= 4-5/group) and analyzed in duplicate. In the case of the IL-13 KO experiments, RNA from individual mouse lungs (n= 3/group) were analyzed. RNA samples were hybridized to Affymetrix Murine Genome U74v2 Gene Chips, containing probe sets interrogating 36,000 full-length mouse genes and EST clusters from the UniGene database (Build 74). Samples were hybridized for 16 hours in an Affymetrix Hybridization Oven 640 (Affymetrix, Santa Clara, CA). The microarray chips were washed and stained on the Affymetrix Fluidics Station 400 using instructions and reagents provided by Affymetrix. This involves removal of non-hybridized material and incubation with phycoerythrin-streptavidin to detect bound cRNA. To amplify the signal intensity, chips were washed and restained with biotin-labeled anti-streptavidin antibody followed by phycoerythrin-streptavidin staining. The stained arrays were

scanned using the Hewlett-Packard G2500A Gene Array Scanner (Hewlett-Packard, Palo Alto, CA) at a wavelength of 488nm.

Microarray Data Analysis: Gene expression summary values for the Affymetrix GeneChip data in CEL files were computed using RMA Express (http://rmaexpress.bmbolstad.com/). All data analyses were carried out with GeneSpring Software version 7.3.1 (Agilent Technologies, Foster City, CA), including filtering, statistical analyses, and hierarchical clustering. Global normalization was performed across all microarrays by adjusting the average intensity of the experimental chips (allergen, cytokine treatments) to the average intensity of their corresponding controls (PBS). Hybridization signals were transformed from log base 2 to linear values and reported as mean fold inductions. Differential gene expression between treatment groups and their corresponding controls was determined via Student's t-test ($p \leq 0.05$). Where specified, the differentially expressed gene sets were further filtered by the criteria of fold inductions ≥ 2 fold.

Quantitative real-time RT-PCR: Total RNA was extracted from frozen lungs using Trizol Reagent® (Invitrogen, Carlsbad, CA) per the manufacturer's protocols, followed by purification using RNeasy mini kit and DNAse digestion (Qiagen, Valencia, CA). RNA purity was confirmed with a Nanodrop® Spectrophotometer (Nanodrop, Wilmington, DE), and RNA integrity was confirmed using a Bioanalyzer (model 2100, Agilent Technologies, Inc., Palo Alto, CA). Purified total lung RNA was reverse transcribed into single-stranded cDNA using random hexamers and Superscript II (Invitrogen, Carlsbad,

CA). Real-time RT-PCR was performed on the iCycler (Roche Diagnostics, Switzerland), using a total volume of 20 µl, containing 100 µM of iCycler-DNA Master SYBR Green (Roche Diagnostics, Mannheim, Germany), ddH_20, and 4 µL of cDNA, corresponding to approximately 33 ng of total RNA. The cDNA was added as template and 5 µl (3mM) of the primer of interest was added to the PCR reaction. PCR primer sequences were selected from the PrimerBank database (http://pga.mgh.harvard.edu/primerbank) and were synthesized by Integrated DNA Technologies (Coralville, IA): *β-Actin*: sense primer, 5'-GTGACGTTGACATCCG -3'; antisense primer, 5'- CAGTAACAGTCCGCCT-3'; *Retnlb*: sense primer, 5'-AGTGAATCTGCTCTTAGG -3'; antisense primer, 5'-ATCCAGTGACAACCATCC-3'; *Agr2*: sense primer, 5'-TTCATCACTTGGACGAATGC -3'; antisense primer, 5'-TGTGTCAGAAGGTTCATAAGC-3'; *Ccl1*: sense primer, 5'-CTGCTCACGGTCACTTCC -3'; antisense primer, 5'-CAAGAGAGGAGGTTGTTTATG-3'; *Sprr2a*: sense primer, 5'-CGACCTTCCTCATTCTTAGC -3'; antisense primer, 5'-TCTCTGTGGCTTATCCTTCC-3'; *Aass*: sense primer, 5'-TCAACAGAGAAGCATACC -3'; antisense primer, 5'-CCTCATCACAATCATATCC-3'; *Itlnb*: sense primer, 5'-GCACCTTCACTGGCTTCC -3'; antisense primer, 5'-GAACACTCTGAACTGAACATATCC-3'; *scinderin:* sense primer 5'CGACGTCGTCACATTGTCC-3'; anti-sense primer, 5'TCATTGTGGTGGAAGAAGGA-3'; *Slc5a1*, 5'CCACAAAGTGACCACTTCCA-3'; anti-sense primer 5'GTGGTACCGTTGGAGGCTT-3'; *IFN-γ*, sense primer 5' TCAAGTGGCATAGATGTGGAAGAA; anti-sense primer, 5'-

TGGCTCTGCAGGATTTTCATG-3'. The amount of mRNA transcripts encoding these genes was determined using the following formula: Relative Gene Expression = $(1.8^{(a-b)})$ x 100,000 where a= crossing point of β-actin or GADPH and b= crossing point of gene of interest.