Supplementary Material

Methods

Microarray analysis

Before microarray hybridization, the quality of the isolated RNA was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA). Double-stranded cDNA was synthesized from total RNA extracted from whole mouse lungs using the SuperScript Choice kit (Invitrogen, Paisly, UK) with a T7- (dT) 24 primer incorporating a T7 RNA polymerase promoter (Metabion, Martinsried, Germany). cRNA was prepared and biotin labeled by *in vitro* transcription (Enzo Biochemical, New York, USA). Labeled RNA was fragmented by incubation at 94°C for 35 min in the presence of 40 mM Tris-OAc (pH 8.1), 100 mM KOAc, and 30 mM MgOAc. Labeled and fragmented cRNA (15 µg) was hybridized for 16 h at 45°C to a MG-U74Av2 mouse genome array (Affymetrix, High Wycombe, UK). After hybridization, the gene chips were automatically washed and stained with streptavidin-phycoerythrin using a fluidics station. Probe arrays were scanned at 3-µm resolution using a Genechip Scanner with confocal optics made for Affymetrix by Agilent (Böblingen, Germany).

Each cRNA sample generated from one lung was hybridized on one MG-74Av2 array. In total, 8 lungs were analyzed, two treated mice vs. two controls in two different mouse strains (BALB/c and C57BL/6) respectively. Affymetrix's Microarray Suite software 5.0 (MAS5), MicroDB, and Data Mining Tool were used to scan and analyze the relative abundance of each gene based on the intensity of the signal from each probe set. Arrays were scaled by MAS5 to a target value of 150 (scaling factor range 0.33-0.96). The data generated from different mouse strains was analyzed separately. We excluded all genes with absent call ($p>0.06$) in all 4 samples from the initial groups. We obtained a list of 7815 probe sets (BALB/c) (62,6 % of genes present on the MG-74Av2 array) and a list of 7290 probe sets (C57BL/6) (58,4 % of genes present on the MG-74Av2 array) respectively.

Two arrays (treated vs. control) comparisons analysis were performed allowing a total of four comparisons (2 × 2 matrix). For each comparison analysis, MAS5 generates a "difference call" of no change, marginal increase/decrease, or increase/decrease, respectively. Only those genes which were found to be similarly regulated in all 4 comparisons were classified as differentially expressed genes. The MAS5 fold-change output of a comparison analysis is the signal log ratio (SLR), which is the fold change presented in log2. The SLR was converted to a standard; no logarithmic scale and the mean fold change of all 4 comparisons was calculated. Gene categorization was based on the NetAffx database (http://www.affymetrix.com/). The output from the microarray analysis was merged with the Unigene or GenBank descriptor and saved as an Excel data spreadsheet.

Real time RT-PCR

PCR amplification and analysis were achieved using an ABI PRISM 7700 (Perkin Elmer, Rodgau, Germany) and SDS software version 1.7. The reaction mixture consisted of 100 ng of total RNA isolated from treated and untreated mouse lung tissue (Invisorb RNA Kit II, Invitek, Germany), 0.5 µM of each specific primer, 0.5 µl RNAsin (40 U/µl, Promega, Manheim, Germany), 0.5 µl MMLV-H reverse transcriptase (200 U/µl, Promega), 1 µl AmpliTaqGold® (5 U/µl, Applied Biosystems, Foster city, CA, USA), 5µl 10 X PCR buffer without $MgCl_2$ (containing 100 mM Tris-HCL pH 8.3, 500 mM KCL), 2 µl $MgCl_2$ (25 mM, Applied Biosystems), 1 µl dNTPmix (10 mM, Applied Biosystems), 0.5 µl SYBRgreen (50 X, Biozym, Germany) and 0.25 µl Rox (150 µM TIPMOLBIOL, Berlin, Germany) in a total volume of 50 µl. All templates were amplified using the following protocol: RT-step was done at 48°C for 30 min, the polymerase (AmpliTaqGold, Applied Biosystems) was activated and cDNA denatured by a preincubation for 10 min at 95°C; the template was amplified for 40 cycles of denaturation for 15 s at 95°C, annealing of primers and extension at 60°C for 1 min. Fluorescence data were acquired during each extension phase. To determine the presence

of contaminating genomic DNA, the reverse transcriptase was omitted as a negative control. As an additional positive control, a primer-pair for ß-actin was used to amplify actin under the same conditions. Product identity was confirmed by electrophoresis on a 4% agarose gel stained with ethidium bromide.

List of primers

Gene	**Species**	**GenBank Acc. No.**	**Forward primer 5´-3´**	**Reverse primer 5´- 3´**	**Amplicon size (bp)**
Gpx2	Mouse	X91864	CCAAGTCGTTCTA CGATCTC	CACATTCTCAATC AGCACAG	95
GSTO	Mouse	AI843119	TAATTTGACCTTC TGGCCTA	GAAAGTATGGGG AAATCACA	212
ß-Actin	Mouse	X03672	GTTTGAGACCTTC AACACCCCA	CTGGTCTCCGTAT GT CCC TGT	71

Protein preparation, SDS-PAGE and Western blotting

One gram of mouse lung tissue (snap-frozen, stored at -80°C) was homogenized in 3 ml ice cold 1 x PBS, pH 7.4, 1% Nonidet P-40 (Fluka), 0.5% Na-Deoxycholate (Roth, Karlsruhe, Germany), 0.1% SDS (Sigma-Aldrich,Germany); 10 µg/ml phenylmethylsulfonyl fluoride (Roth); 50 KIU/ ml aprotinin (Santa Cruz Biotech); 1mM/ml (activated) sodium orthovanadate (Sigma-Aldrich,Germany). The supernatants were cleared twice by centrifugation at 10000 x g for 15 minutes at 4°C. Proteins were separated by SDS-PAGE (12% acrylamide gel) and transferred to a nitrocellulose membrane (Schleicher & Schuell, Germany). After transfer , the membrane was blocked and incubated with primary antibodies (both anti-*Gpx-2* or anti-GSTO 1-1 at 1:1000 dilution) overnight at 4ºC, then washed and incubated with HRP-conjugated secondary antibody (1:10000, Amersham Pharmacia Biotech, Freiburg, Germany for 1h at RT. The blot was developed using a chemiluminescence

detection kit (ECL, Amersham Life Science, Little Chalfont, UK) according to the manufacturer's instructions.

Data

Table Is: Kinetic of differential cell count in BAL fluid at 16h and 48h after last allergen challenge

	16h control	**16h OVA**	**48h control**	**48h OVA**	
Celltyp	2.2	258	0.001	2.2	x 10^3/ ml neutrophils
	n/d	n/d	0.1	50.4	x 10^3/ ml eosinophils
	n/d	n/d	0.07	11.0	x 10^3/ ml lymphocytes
	n/d	n/d	21.6	28.6	x 10^3/ ml macrophages

(n/d: not detectible)

Table IIs: List of up regulated genes after OVA challenge

Identifier	Gene Title	Gene symbol	BALB/c (fold-change)	C57BL/6 (fold-change)
EDL04793	arginase 1, liver	Arg1	66,2 ± 15,8	13,4 ± 1,6
BE200119	small chemokine (C-C motif) ligand 11	Ccl11	14,2 ± 6,5	2,8 ± 0,4
BC145867	chemokine (C-C motif) ligand 2	Ccl2	9,9 ± 0,4	11,9 ± 1,9
L04694	chemokine (C-C motif) ligand 7	Ccl7	39.4 ± 6.2	20.4 ± 7.6
AAF22536	chemokine (C-C motif) ligand 9	Ccl9	3.5 ± 0.6	2.8 ± 0.2
AAH39919	cholesterol 25-hydroxylase	Ch25h	3.8 ± 0.2	3.3 ± 0.5
U56900	chitinase 3-like 3	Chi3l3	4,8 ± 0,5	2,6 ± 0,4
BC011134	chitinase, acidic	Chia	35.8 ± 13.9	3.2 ± 0.1
BC116319	chloride channel calcium activated 3	Clca3	65,8 ± 35,0	32,5 ± 1,1
AAD15798	Cytokine-responsive protein CR6	CR6	4.0 ± 0.7	2.1 ± 0.1
AK180750	esterase D/formylglutathione hydrolase	Esd	2.4 ± 0.1	2.5 ± 0.2
AK170282	coagulation factor X	F10	2.3 ± 0.4	2.3 ± 0.3
BG087011	glycine amidinotransferase	Gatm	2.4 ± 0.2	2.7 ± 0.5
BC010823	**glutathione peroxidase 2**	**Gpx2**	7.9 ± 2.9	2.6 ± 0.2
BAE27469	**glutathione S-transferase omega 1**	**Gsto1**	4.0 ± 0.3	2.5 ± 0.2
AK085689	potassium inwardly-rectifying channel	Kcnj15	180.3 ± 54.7	2.1 ± 0.3
AAI32070	lipocalin 2	Lcn2	9.8 ± 0.5	3.4 ± 0.3
BAE42486	matrix metallopeptidase 12	Mmp12	4,2 ± 0,8	4,5 ± 1,1
AAH61139	regenerating islet-derived 3 gamma	Reg3g	45.7 ± 15.7	2.6 ± 0.3
AAH55885	serum amyloid A 3	Saa3	10.9 ± 0.6	57.1 ± 6.9
AAA40130	serine peptidase inhibitor, member 3N	Serpina3n	5.8 ± 0.6	3.3 ± 0.5
AF208031	solute carrier family 5, member 1	Slc5a1	19.7 ± 17.4	2.7 ± 0.2
CAM19105	suppressor of cytokine signaling 3	Socs3	2,3 ± 0,1	2,1 ± 0,3
AK007633	small proline-rich protein 2A	Sprr2a	8.9 ± 1.8	2.5 ± 0.7

AK007296	sulfiredoxin 1 homolog	Srxn1	9.4 ± 3.0	2.1 ± 0.1
BC008107	tissue inhibitor of metalloproteinase 1	Timp1	5.7 ± 1.2	2.5 ± 0.3
BC004676	upstream binding transcription factor	Ubtf	5.3 ± 0.7	2.3 ± 0.1