Online supplementary file

Online supplementary methods

Measurement of serum cytokines: limit of detection (LOD)

The concentrations are LOD/2 of 4-fold diluted serum samples

	Plate 1	Plate 2
IL-1β	0.3	0.2
IL-2	1	0.9
IL-4	0.08	0.1
IL-5	0.2	0.3
IL-6	0.3	0.3
IL-8	0.7	2.7
IL-9	0.4	0.5
IL-10	1.6	1.4
IL-12 (p70)	1.1	1.1
IL-13	0.8	0.75
IL-17A	1.7	1.2
IL-17F	3.4	4.3
IL-22	0.6	0.9
IL-23	2.9	11.1
IL-33	2	1.9
Eotaxin-1	0.4	1.5
IFN-γ	11.4	1.6
MIP-1α	0.3	0.3
MIP-1β	0.8	0.8
TNF-α	1.4	1.5

Statistics

The study was cross-sectional. Statistical analysis was performed using SAS 9.4 statistical software (SAS Institute, Cary, North Carolina, USA). Variable distributions were examined for normality, and non-parametric tests or logarithmic transformation used when appropriate (χ^2, Fisher's exact, Wilcoxon two-sample, or Kruskal-Wallis test, analysis of variance, Spearman's rank correlation coefficient). We used the Hodges-Lehmann estimation of the location shift for two-sample data and asymptotic confidence limits for the location shift. Subjects with a missing value for one variable were excluded from the analyses using this variable. Results <LOD were included in the calculations and were not considered as missing.

Serum cytokine concentrations were used for performing hierarchical clustering analyses (HCA) generated with the TM4 Multiexperiment Viewer (Saeed AI et al. 2003) (MeV v4.8, Dana-Farber Cancer Institute, Boston, USA). Cluster trees were created using the Spearman rank correlation as distance metric with average linkage clustering.

Multiple linear regression models were used to identify determinants of cytokine concentrations. For selecting the variables that should be included in the models, the Bayesian information criterion (BIC), Akaike's information criterion (AIC) and the explanatory value (adjusted R^2) were used as goodness-of-fit statistics. If the information gained by BIC, AIC and adjusted R^2 value did not indicate the same, the BIC was used to decide which model is best because it prefers smaller models which have the same explanatory value than larger ones. Similarly, if BIC values of different models were within 1-2 units the simpler model was preferred. In each model the assumptions of linear regression and collinearity of the covariates and intercept were assessed.

As the a priori defined linear regression model (including age, sex, atopy and, using the Sy-/Se- subgroup as the reference, three dummy variables for the three remaining combinations of symptoms and sensitization) proved unsatisfactory, new models were developed for each cytokine on the basis of these preliminary results and additional systematic bivariate analyses. The dummy variable(s) defining the combinations of symptoms and sensitization were kept in every model. Additional variables (e.g. atopy, allergy history, drug consumption, current rhinitis, recent symptoms, smoking, BMI) were included in the model after considering biological knowledge and/or the results of the bivariate analyses using a p-value >0.20 as a cut-off. These variables were added one by one, evaluating their effect on the aforementioned goodness-of-fit criteria. Cytokines playing a crucial role in asthma and inflammation were included in the models as appropriate (IL-13 as a master Th2-related cytokine; IL-1β and TNF-α as master pro-inflammatory cytokines). Decision about these three cytokines was made on the basis of pathophysiological knowledge before examining the data. In this way, nine regression models were examined for most individual cytokines in the final statistical analyses. P-values <0.05 were considered statistically significant and were reported without correction for multiple testing.

Data on prevalence and incidence of LAA show considerable discrepancies making accurate power calculations impossible. Therefore, crude power calculations in the study protocol were based on the study by Pacheco KA et al. (2003) that was the most recent study having examined the four possible combinations of symptoms and sensitization simultaneously. Using cytokine profiles as a categorical variable and assuming that 20% of the subjects without occupational animal allergy would have a false-positive IgE test but 20% of those with allergy would have a false-negative IgE test, a comparison of subgroups Sy-/Se- and Sy+/Se+ would require (p-value of 1%, power of 90%, subgroup ratio of 6 to 1) about 66 and 11 subjects, respectively, to detect a clinically significant difference. Similar calculations show that two groups of 14 subjects are required to have enough power for detecting a clinically meaningful difference in cytokine profiles between subgroups Sy+/Se+ and Sy+/Se-. Therefore, a population of ≥30 symptomatic workers (15 with and 15 without sensitization) and 70 reference workers was deemed acceptable. When using the aforementioned categorization scheme, prevalence proportions of Sy+/Se+, Sy+/Se-, and Sy-/Se+ subjects of 10 to 13% can be expected (Pacheco KA et al. 2003). Hence, a total population of at least 150 LA workers was aimed at to have about 15 workers each in the Sy+/Se+, Sy+/Se-, and Sy-/Se+ subgroups.

References

Pacheco KA, Mccammon C, Liu AH et al. Airborne endotoxin predicts symptoms in non-mouse-sensitized technicians and research scientists exposed to laboratory mice. Am J Respir Crit Care Med 2003;167(7):983-990.

Saeed AI, Sharov V, White J et al. TM4: a free, open-source system for microarray data management and analysis. Biotechniques 2003;34(2):374-378.

Current working conditions

Workers with cytokine measurements (n=114) were from a study, the purpose of which was to inform updated surveillance and prevention measures. Accordingly, the current exposure to airborne rat and mouse allergen and endotoxin and the prevalence of sensitization to rat and/or mouse were assessed. Personal sampling (March to July 2014) was used to collect samples in a subpopulation selected according to predefines rules. As a rule, sampling was carried out separately in the morning and afternoon. The mean of the morning and afternoon measurement of each worker and the highest of the individual morning and afternoon measures was defined as the daily and the maximum exposure, respectively. As briefly summarized in the present publication, results showed low or non-detectable airborne concentrations of allergens and endotoxin. Moreover, endotoxin proved to be a minor cause

of symptoms and on the basis of occupational and clinical history various other reasons for being “symptomatic” were identified. Clinical and occupational history suggested that recent sensitization to mice and/or rat was a rare event. A comprehensive description of the endotoxin and allergen measurements and a detailed presentation of the sensitized and/or symptomatic groups will be published elsewhere (Oppliger A et al.; in revision).

Online supplementary results

Serum cytokine concentrations of the outlier

One single worker had several cytokine concentrations greatly exceeding those found in the rest of the population in the absence of any identifiable cause and was excluded. The excluded worker belonged to the Sy+/Se- subgroup. Her serum concentrations were 41.2, 508.8, 1466.9, 1739.1, 58.5, 91.7, 552.1, 2341.3, 849.8, 828.7, 10.1, 107, 14 510.1 for IL-4, IL-5, IL-13, eotaxin-1, IL-8, IL-17A, IL-1β, IL-9, IL-10, IL-12, MIP-1α, MIP-1β, and TNF-α, respectively. Concentrations of IL-17F, IL-22, IL-23, and IL-33 were all <LOD.

Online supplementary table S1 Characteristics of the study population

	GROUP Sy-/Se- (n=71)	**Sy+/Se+ (n=8)**	**Sy+/Se- (n=29)**	**Sy-/Se+ (n=6)**
Age (years)	29.0 (16-46)	50.0 (27-64)	31.0 (22-58)	39.0 (28-53)
Sex (women)	51 (71.8)	4 (-)	18 (62.1)	3 (-)
Education level				
High	55 (77.5)	7 (-)	20 (69.0)	5 (-)
Middle	15 (21.1)	1 (-)	8 (-)	1 (-)
Low	1 (-)	0 (-)	1 (-)	0 (-)
Nationality				
Switzerland	28 (39.4)	4 (-)	12 (41.4)	1 (-)
Germany	14 (19.7)	1 (-)	11 (37.9)	2 (-)
Italy	10 (14.1)	0 (-)	1 (-)	0 (-)
Other	19 (26.8)	3 (-)	5 (-)	3 (-)
Smoking				
Never	50 (70.4)	5 (-)	23 (79.3)	3 (-)
Former	8 (-)	2 (-)	4 (-)	2 (-)
Current	13 (18.3)	1 (-)	2 (-)	1 (-)
Pack-years (cigarette smokers only) (n)	2.10 (0.05-14) (21)	4.05-35 (3)	0.25-15.0 (6)	0.20-5.25 (3)
BMI (kg/m^2)	22.3 (17.3-36.0)	25.2 (19.4-35.3)	23.3 (16.4-35.4)	23.2 (21.5-32.8)
FEV1 (percent predicted)	103.1 (80.3-128.5)	98.5 (84.3-109.1)	103.1 (68.9-136.9)	94.1 (87.4-128.2)
FEV1/FVC (%)	79.3 (63.7-94.9)	70.1 (59.2-80.6)	78.0 (58.2-90.1)	79.3 (71.5-80.5)
Health condition				
"Symptomatic"				
No	23 (32.4)	0 (-)	0 (-)	0 (-)
Non-WR	48 (67.6)	0	0	6 (-)
WR	0 (-)	8 (-)	29 (100)	0 (-)
Allergic rhinitis				
No	49 (69.0)	1 (-)	11 (37.9)	5 (-)

NWR	22 (31.0)	0 (-)	8 (-)	1 (-)
WR	0 (0)	7 (-)	10 (34.5)	0 (0)
Any allergic disease				
No	36 (50.7)	0 (0)	3 (-)	1 (-)
NWR	35 (49.3)	1 (-)	2 (-)	5 (-)
WR	0 (0)	7 (-)	24 (82.8)	0 (0)
Asthma				
No	57 (80.3)	1 (-)	21 (72.4)	3 (-)
NWR	14 (19.7)	1 (-)	4 (-)	3 (-)
WR	0 (-)	6 (-)	4 (-)	0 (0)
Total IgE (kU/l)	28.1 (1.00-1104)	163.5 (22.5-1209)	63.3 (1.00-1127)	146.5 (18.9-306)
Total IgE				
≤100	58 (81.7)	2 (-)	19 (65.5)	3 (-)
>100	13 (18.3)	6 (-)	10 (34.5)	3 (-)
Specific IgE (≥0.35 kUA/l)				
Rat	0	1	0	0
Mouse	0	1	0	4
Mouse + rat	0	6	0	2
Lifelong occupational exposure to animals (years)	3.0 (0-9)	20.5 (3-39)	7.0 (0-37)	10.5 (4-32)
Exposure in last job (years)	2.0 (0-9)	4.0 (0-34)	2 (0-19)	5 (1-14)

Values are median and range or number and percent. n: sample size. One case is missing (no attribution to a subgroup because of missing values). Spirometric results includes only acceptable spirometric curves in Caucasians. Regarding FEV1 (FEV1/FVC), subgroup size is 56 (53), 7 (6), 28 (28), and 5 (5) in subgroups Sy-/Se-, Sy+/Se+, Sy+/Se-, and Sy-/Se+, respectively. “Symptomatic”, “non-work-related” (NWR) and “work-related” (WR): see methods. Note that according to the classification scheme subjects having any pre-defined symptom are considered asymptomatic when the symptom is not work-related. “Other nationality” includes >7 different nationalities
Any allergic disease comprises allergic rhinitis, asthma, eczema or any other skin allergy, and allergy to animals
Asthma (occupational and/or non-occupational) had been confirmed by a doctor in 28/32 (87.5%) workers.
Cases who were both WR (work-related) and non-WR were considered as WR.

Online supplementary table S2 Serum cytokine concentrations (pg/ml) after excluding the outlier (n=113)

Subgroup	**Sy-/Se- (n=71)**	**Sy+/Se+ (n=8)**	**Sy+/Se- (n=28)**	**Sy-/Se+ (n=6)**	**p-value (all subgroups and Sy-/Se- vs Sy+/Se-) ‡**
	Median (range) †	*Median (range) †*	*Median (range) †*	*Median (range) †*	
Cytokines selected according to the a priori hypothesis					
"Allergy" profile					
IL-4	9.28 (4.52-14.44)	7.80-11.32	9.80 (6.88-13.40)	7.40-23.52	0.32/0.13
IL-5	3.84 (0.80-15.84)	0.80-8.92	4.72 (1.88-7.12)	3.24-5.96	0.01/0.004
IL-13	16.68 (3.20-58.52)	10.20-38.00	16.64 (10.40-28.20)	11.36-28.20	0.50/0.94
Eotaxin-1	46.00 (22.48-131.88)	33.88-124.32	39.38 (30.40-89.48)	30.40-56.44	0.28/0.14
"Irritation" profile					
IL-8	19.28 (8.24-54.36)	10.80-41.48	22.78 (10.80-255.68)	10.80-89.00	0.19/0.99
IL-17A	20.72 (6.80-213.56)	15.84-316.76	40.16 (4.80-163.92)	24.36-51.60	0.003/0.001
IL-17F	All concentrations <LOD				
IL-22	109/113 concentrations <LOD				
Cytokines selected for explorative analyses					
Cytokines related to the "allergy" profile					
IL-9	33.68 (8.44-129.16)	26.52-55.48	24.90 (11.28-136.00)	15.48-45.32	0.002/0.005
IL-33	106/114 concentrations <LOD				
Cytokines related to the "irritation" profile					
IL-1β	1.84 (0.44-12.08)	1.64-11.00	2.32 (1.20-3.84)	1.20-2.76	0.01/0.01
TNF-α	26.88 (5.60-116.80)	24.24-507.84	24.24 (13.44-131.68)	18.84-37.64	0.18/0.47
IL-23	84/114 concentrations <LOD. Moreover, 16 of the 30 concentrations >LOD were the same (196.8 pg/ml)				
IL-12	66.76 (4.40-200.84)	24.20-200.92	41.08 (17.80-237.36)	30.12-123.48	0.009/0.002
IL-10	14.04 (3.60-269.00)	7.20-56.32	8.86 (4.36-41.36)	6.68-31.84	0.005/0.003
MIP-1α	4.84 (2.56-9.04)	4.56-20.92	4.10 (2.80-7.04)	3.92-5.56	0.08/0.04
MIP-1β	102.28 (43.24-224.56)	68.96-175.24	78.56 (33.88-239.32)	29.72-145.28	0.04/0.01
Cytokines selected for comparison with the literature					
IL-2	4.00 (4.00-265.64)	4.00-84.40	6.92 (3.60-39.48)	3.60-111.00	0.03/0.16
IL-6	8.84 (2.52-84.16)	6.96-71.68	9.76 (2.96-46.64)	6.84-18.12	0.78/0.59
INF-γ	229.88 (45.60-1534.64)	229.88-1167.72	248.10 (56.68-563.12)	133.44-480.40	0.12/0.73

* Cytokine serum concentrations of the excluded worker: see above "*Serum cytokine concentrations of the outlier"*.
† Median is not indicated when group size is <10.
‡ p-value of difference: The first and the second level of significance were calculated between all subgroups (Kruskall-Wallis test) and subgroups Sy-/Se- and Sy+/Se- only (Wilcoxon two-sample test).

Online supplementary table S3 cytokines selected a priori without exclusion of the outlier

	IL-4	IL-5	IL-13	eotaxin-1	IL-8	IL-17A
Sample size	n=114	n=114	n=111	n=114	n=114	n=114
Concentrations <LOD	n=0	n=16	n=7	n=0	n=15	n=21
Intercept	**0.608** **<0.0001**	**-0.548** **<0.0001**	**1.033** **<0.0001**	**1.651** **<0.0001**	**1.220** **<0.0001**	**0.146** **<0.0001**
Sy+/Se+ subgroup	0.003 0.92	0.065 0.47	0.149 0.21	0.051 0.50	-0.027 0.75	0.222 0.07
Sy+/Se- subgroup	0.018 0.33	**0.137** **0.01**	0.108 0.08	-0.006 0.89	0.037 0.46	**0.181** **0.01**
Sy-/Se+ subgroup	0.067 0.06	0.159 0.12	0.097 0.42	-0.039 0.64	**0.219** **0.02**	0.216 0.12
Drug consumption	0.006 0.71	0.002 0.96	**0.128** **0.02**	0.070 0.07	NI	0.112 0.07
Recent complaints	NI	**0.110** **0.02**	**0.138** **0.01**	**0.094** **0.01**	NI	NI
Sex	NI	NI	NI	-0.079 0.06	NI	NI
IL-13	**0.290** **<0.0001**	**0.807** **<0.0001**	NR	NR	NR	NR
IL-1β	NR	NR	NR	NR	**0.269** **0.0004**	**0.503** **<0.0001**
Adjusted R^2	**0.53**	**0.56**	**0.10**	**0.06**	**0.13**	**0.28**
Pr > F	<**0.0001**	**<0.0001**	**0.008**	**0.05**	**0.0006**	**<0.0001**

Values are partial regression coefficients and significance levels. LOD: limit of detection. NI: variable not included in the model with the best fit statistics. NR: not relevant in this case (see methods). Serum cytokine concentrations were log transformed. As dummy variables were used, regression coefficients indicate the difference between the asymptomatic and non-sensitized subgroup which was the reference and each specific subgroup.
n=111: Three cases with missing values for one independent variable were excluded from all models relating to IL-13.
Drug consumption: regular consumption of any drug. Recent complaints: see methods.

Rationale for including IL-1β and IL-13: see methods. Concentrations of IL-17F and 22 are not presented because on the high percentage of concentrations <LOD.
Pr > F: p-value of an F-test, testing the null hypothesis that all model coefficients are zero.

Online supplementary table S4 Multiple linear regression analyses: cytokines selected a priori after excluding the outlier

	IL-4	IL-5	IL-13	eotaxin-1	IL-8	IL-17A
Sample size	n=113	n=113	n=110	n=113	n=113	n=113
Concentrations <LOD	n=0	n=16	n=7	n=0	n=15	n=21
Intercept	**0.635** **<0.0001**	**-0.356** **0.003**	**1.065** **<0.0001**	**1.710** **<0.0001**	**1.182** **<0.0001**	**1.013** **<0.0001**
Sy+/Se+ subgroup	0.006 0.84	0.089 0.31	0.144 0.12	0.085 0.09	-0.059 0.50	0.104 0.36
Sy+/Se- subgroup	0.017 0.35	**0.130** **0.01**	0.047 0.33	-0.031 0.29	0.034 0.51	**0.166** **0.02**
Sy-/Se+ subgroup	**0.068** **0.05**	0.171 0.08	0.086 0.35	-0.020 0.71	**0.212** **0.03**	0.185 0.14
Drug consumption (0=no; 1=yes)	0.007 0.67	0.010 0.82	**0.094** **0.03**	**0.058** **0.02**	NI	0.094 0.10
Recent complaints (0=no; 1=yes)	NI	**0.121** **0.009**	**0.108** **0.01**	**0.088** **0.0006**	NI	NI
Sex (0=male; 1=female)	NI	NI	NI	**-0.096** **0.0003**	NI	NI
History of allergic disease (0=no; 1=yes)	NI	NI	NI	**-0.076** **0.009**	NI	NI
IL-13 (pg/ml)	**0.266** **<0.0001**	**0.634** **<0.0001**	NR	NR	NR	NR
IL-1β (pg/ml)	NR	NR	NR	NR	**0.412** **0.0004**	**1.045** **<0.0001**
Adjusted R^2	**0.36**	**0.38**	**0.08**	**0.21**	**0.13**	**0.39**

Pr > F	<**0.0001**	**<0.0001**	**0.02**	**<0.0001**	**0.0009**	**<0.0001**	

Values are partial regression coefficients and significance levels. LOD: limit of detection. NI: variable not included in the model with the best fit statistics. NR: not relevant in this case (see methods). Serum cytokine concentrations were log transformed. As dummy variables were used, regression coefficients indicate the difference between the asymptomatic and non-sensitized subgroup which was the reference and each specific subgroup.
n=110: Three cases with missing values for one independent variable were excluded.
Drug consumption: regular consumption of any drug. Recent complaints and history of allergic disease: see methods.
Rationale for including IL-1β and IL-13: see methods. Concentrations of IL-17F and 22 are not presented because on the high percentage of concentrations <LOD.
Pr > F: p-value of an F-test, testing the null hypothesis that all model coefficients are zero.

Online supplementary table S5 cytokines selected for exploratory analyses after excluding the outlier

	IL-1β	IL-9	IL-10	IL-12	MIP-1α	MIP-1β	TNF-α
Sample size	n=113	n=113	n=110	n=110	n=113	n=110	n=110
Concentrations <LOD	n=0	n=0	n=7	n=2	n=0	n=0	n=2
Intercept	**0.178** **<0.0001**	**1.454** **<0.0001**	**1.066** **<0.0001**	**1.626** **<0.0001**	**0.663** **<0.0001**	**2.011** **<0.0001**	**1.193** **<0.0001**
Sy+/Se+ subgroup	**0.236** **0.0006**	0.072 0.34	0.105 0.36	0.022 0.85	**0.088** **0.03**	0.021 0.78	0.038 0.54
Sy+/Se- subgroup	0.074 0.07	**-0.103** **0.02**	-**0.168** **0.006**	**-0.149** **0.02**	-0.036 0.13	**-0.096** **0.02**	**-0.066** **0.03**
Sy-/Se+ subgroup	0.076 0.32	-0.108 0.19	-0.117 0.31	-0.038 0.75	-0.011 0.80	**-0.153** **0.04**	-0.028 0.63
Drug consumption (0=no; 1=yes)	**0.069** **0.04**	0.064 0.08	**0.159** **0.003**	**0.144** **0.008**	**0.039** **0.05**	NI	NI
Recent complaints (0=no; 1=yes)	**0.107** **0.002**	NI	NI	**0.147** **0.008**	NI	NI	NI
Total IgE (kU/l)	NI	0.0002 0.09	NI	NI	NI	NI	NI
IL-1β (pg/ml)	NR	NI	NI	NI	NI	NI	**0.857** **<0.0001**

Adjusted R^2	**0.16**	**0.10**	**0.13**	**0.11**	**0.09**	**0.06**	**0.60**
Pr > F	**0.0002**	**0.008**	**0.0008**	**0.003**	**0.008**	**0.03**	**<0.0001**

Values are partial regression coefficients and significance levels. NI: variable not included in the model with the best fit statistics. Serum cytokine concentrations were log transformed. As dummy variables were used, regression coefficients indicate the difference between the asymptomatic and non-sensitized subgroup which was the reference and each specific subgroup.

IL-10: Models with regulatory cytokines (IL-1β and TNF-α) not considered because of collinearity.

MIP-1α and MIP-1β: Models with the two regulatory cytokine (IL-1β and TNF-α) excluded because of collinearity.

n=110: Three cases with missing values for one independent variable excluded from all models.

Drug consumption: regular consumption of any drug with the exception of MIP-1α for which occasional consumption of drugs possibly altering cytokine metabolism was statistically significant (see methods). Recent complaints: see methods.

Concentrations of IL-23 and IL-33 are not presented because on the high percentage of concentrations <LOD.

Pr > F: p-value of an F-test, testing the null hypothesis that all model coefficients are zero.