Associations between pre-employment immunologic and airway mucosal factors and the development of occupational allergy

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Background: Sensitization to occupational allergens is frequently found in laboratory animal workers (LAWs) and can cause serious health problems. Atopy is a major risk factor for sensitization, but it is considered insufficient to advise against working with animals.

Objective: We investigated whether immunologic measures, including serology and cytokine production profiles of blood cells, and parameters for airway inflammation are associated with the development of occupational sensitization.

Methods: In a prospective cohort study 110 starting LAWs were followed for 2 years. At inclusion, results of health questionnaires, skin test results, lung function measures, methacholine threshold levels, and nasal lavage fluid were obtained. Blood was taken for measuring total IgE and allergen-specific IgE antibodies. Cytokine production profiles were measured in whole blood.

Results: Twenty-two new cases of sensitization were identified during follow-up. In multivariate logistic regression analysis a model including atopy and total IgE level predicted sensitization best. This was corroborated in a separate validation cohort. Parameters for airway inflammation or cytokine production profiles did not further contribute to the prediction of sensitization. Based on these results, pre-employment counseling aimed at applicant LAWs with atopy and a total IgE level of greater than 100 IU/mL might be able to reduce occupational sensitization by up to 45% to 50% with less than 10% false-positive predictions.

Conclusion: The combination of atopy and total IgE level offered the best model to predict development of occupational sensitization. Other immunologic parameters and parameters of airway inflammation did not contribute significantly. (J Allergy Clin Immunol 2009;123:694-700.)

Key words: *Occupational allergy, atopy, IgE, laboratory animal allergy, sensitization*

Work-related allergic symptoms are an important health problem among laboratory animal workers (LAWs). Exposure to rodent allergens in an occupational setting was shown to lead to allergy in 9% to 30% of the employees.[1-3] About 70% of the allergic workers had allergy within the first 2 to 4 years of exposure. Prolonged exposure can eventually lead to occupational asthma in up to one third of sensitized individuals.[1,4]

Several risk factors for the development of laboratory animal allergy have been identified, predominantly in cross-sectional studies. However, interpretation of data from cross-sectional studies is hampered by the "healthy worker effect," a term that refers to a trend toward natural selection of employees without symptoms in specific occupations. This most likely results in an underestimation of the occurrence of work-related problems. The main risk factor for laboratory animal allergy appeared to be atopy.[2,3] Atopy, which is defined as an increased propensity to mount an IgE antibody response to low-dose environmental aeroallergens, is generally established by means of detection of IgE antibodies to common environmental allergens, such as pollen and house dust mite. Depending on the definition of laboratory animal allergy and the design of the studies, atopic subjects were found to be up to 12 times more likely to have laboratory animal allergy. Atopy might also accelerate the development of allergic sensitization and symptoms. Although atopy appears to be the main risk factor for occupational allergy, establishing atopy is generally considered inadequate for pre-employment selection of LAWs because up to 45% of the population in industrialized countries is atopic[5] and the majority of atopic subjects will not become sensitized.[3,6] The rate of false-positive predictions based on atopy is generally well above 20%. Therefore it is generally agreed that the mere presence of atopy is insufficient to advise job applicants against working with allergens.[7] The ability of a predictive algorithm to identify employees at risk within the atopic group would increase the applicability of pre-employment screening and counseling.

In a prospective study with 2 years' follow-up in 110 starting LAWs, we aimed to identify additional pre-exposure immunologic and airway mucosal factors that are associated with the occurrence of occupational sensitization to laboratory animals (SLA). We tried to create a multivariate algorithm that could

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Abbreviations used
LAW: Laboratory animal worker
ROC: Receiver operating characteristic
SLA: Sensitization to laboratory animals

predict occupational sensitization with a less than 10% false-positive rate in employees who will not become sensitized within the first 2 years of exposure but would be able to identify a significant proportion of employees who will become sensitized.

METHODS

Study design and characteristics

In this prospective longitudinal cohort study 110 starting LAWs working with rats, mice, or both were followed for 2 years. The protocol was approved by the Institutional Review Board of the Academic Medical Center of the University of Amsterdam. Written informed consent was obtained from all participants. At inclusion, participants should have had less than 18 months of occupational contact with animals and no sensitization to the animals with which they were working.

The outcome of the study was new sensitization for occupational allergens defined by development of a positive skin prick test response ($\geq$3 mm) or a positive RAST result ($\geq$0.35 IU/mL) for rat or mouse urinary proteins.

Questionnaire results, serologic data, skin test results, airway inflammation measures, and permeability results were obtained for all participants. *In vitro* cytokine production by blood cells was analyzed on a case-control basis. All employees who became sensitized during the 2-year follow-up were compared with a similar number of nonsensitized control subjects matched for atopic status. Parameters available at baseline were analyzed for an association with development of occupational sensitization during follow-up.

Data from a previous observational cohort of 160 LAWs[8] were used for validation of the results with serologic parameters found in the present study. Only questionnaire data, skin test results with environmental and occupational allergens, and total serum IgE levels (quantified with a different assay, Pharmacia Diagnostics AB, Uppsala, Sweden) were available. The mean time of occupational exposure study was variable.

Questionnaire

Participants completed a previously described questionnaire on occupational allergy.[9] Questionnaires were distributed before each visit and provided detailed information on contact with animals, occupational and nonoccupational symptoms, smoking, allergic airway symptoms to environmental allergens, family history of allergy and asthma, pets at home, and animal contact during previous jobs or education.

Mucosal inflammation and permeability

Nasal lavage was performed as described by Lopuhaa et al.[10] Supernatants were stored at −20°C until analysis for the presence of myeloperoxidase, eosinophil cationic protein, α-2-macroglobulin, albumin, and IL-8. These assays were performed as described previously.[10-12]

Lung function and bronchial hyperresponsiveness were measured according to previous guidelines.[13] FEV_1 and forced vital capacity values were expressed as percent predicted. None of the participants had a baseline FEV_1 of less than 65% of the predicted value. Methacholine challenges were performed as described previously.[14] The cumulative dose causing a 15% decrease in FEV_1 was calculated. For analysis, all participants with a threshold exceeding the highest dose administered were allotted a cumulative dose causing a 15% decrease in FEV_1 of twice that dose.

Skin prick testing

Seven common environmental allergens (house dust mite, grass pollen, tree pollen, cat dander, dog dander, rabbit dander, and guinea pig dander), 2 occupational allergens (rat and mouse urine), a positive control (histamine, 10 mg/mL), and a negative control were used for skin prick testing. All common allergens and controls were obtained from ALK-Abelló (Nieuwegein, The Netherlands). Rat and mouse skin tests were obtained as previously described.[2] Skin prick test results were read after 15 minutes and were considered positive if the largest wheal diameter was at least 3 mm and surrounded by erythema. Additionally, results of the negative control test were considered negative when the wheal diameter was less than 1 mm in the absence of erythema.

IgE assays

Blood was taken at each visit, and sera were stored at −20°C until analysis. Total IgE levels were measured in the first sample and quantified as described previously.[15]

Specific IgE antibodies against common allergens and rat urine and mouse urine were determined by using RASTs, as previously described,[16] and expressed in international units of IgE per milliliter.[17] Levels of specific IgE of greater than 0.35 IU/mL were considered positive.

Whole blood culture

Heparin-containing blood was collected at visit A and diluted 10 times in Iscove modified Dulbecco medium (BioWhittaker, Verviers, Belgium) supplemented with 0.1% FCS and 30 U/mL heparin (Leo Pharmaceutical products B.V., Weesp, The Netherlands). Blood was stimulated with *Staphylococcus aureus* Cowan I strain (75 μg/mL; Calbiochem, Darmstadt, Germany) and LPS (1 μg/mL; Sigma-Aldrich, St Louis, Mo) in the absence and presence of recombinant IFN-γ (100 U/mL; U-Cytech, Utrecht, The Netherlands) at 37°C. The supernatant was harvested after 24 hours and stored at −20°C until analysis.

Cytokine levels in supernatants of patients and control subjects were determined with Bioplex assays for IL-4, IL-6, IL-10, IL-12, and IFN-γ (Bio-Rad Laboratories, Hercules, Calif). Assays were performed according to the manufacturer's protocol.

Exposure

For the purpose of characterization of the cohort, mean time of monthly exposure was registered, and individual exposure to occupational allergens was estimated by means of personal air sampling. The method for sampling, eluting, and allergen detection was previously described by Hollander et al.[18] Concentrations were expressed in nanograms of equivalent animal urinary proteins per cubic meter.[18]

The total hours of animal work per period, obtained through questionnaires, were multiplied by the mean exposure measured to obtain a personalized score for estimated accumulated exposure to animal allergens.

Statistical analysis

SPSS version 14.0 software (SPSS, Inc, Chicago, Ill) was used for statistical analysis. Exposure outcomes, methacholine thresholds, and antibody levels were evaluated in terms of their log values. Values of less than the detection limit were allotted half the detection limit. The Student *t* test, Mann-Whitney test, and χ^2 test were used to identify differences between subsets. Parametric correlations between variables were expressed as the Pearson *r* value. To predict SLA, we fitted a multivariate regression model that included all potential predictors with a univariate *P* value of less than .10. Then a backward stepwise procedure was used to select a final model with the strongest predictors for sensitization. A stepwise forward procedure including all parameters with a univariate *P* value of less than .10 was performed for confirmation. Calibration of the model was assessed graphically and tested with the Hosmer-Lemeshow test ($P > .10$ reflects good agreement). The discriminative ability was established with the area under the receiver operating characteristic (ROC) curve. The area under the ROC curve shows the relation between the false-positive rate (1-specificity) and the true-positive rate (sensitivity). The ROC area ranges from 0.5 (no discrimination) to 1.0 (perfect

TABLE I. Characteristics of the study population

	Cohort (n = 110)	Nonatopic subjects (n = 67)	Atopic subjects (n = 43)
Characteristics of the cohort			
Age (y), mean (range)	26 (18-43)	26 (18-40)	26 (20-43)
Sex (male/female)	50/60	31/36	19/24
Smoking (smoker/exsmoker)	18/13	11/8	7/5
At least 1 parent with allergies, no. (%)	54 (49)	29 (43)	25 (58)
Pets at home, no. (%)	60 (55)	38 (57)	22 (51)
Job description			
Rat handler, no. (%)	35 (32)	22 (33)	13 (30)
Mouse handler, no. (%)	57 (52)	35 (52)	22 (51)
Rat and mouse handler, no. (%)	18 (16)	10 (15)	8 (19)
(Post) doctoral research associate, no. (%)	8 (7)	4 (6)	4 (9)
PhD student, no. (%)	66 (60)	41 (61)	25 (58)
Biotechnician, no. (%)	30 (27)	19 (28)	11 (26)
Animal caretaker, no. (%)	6 (5)	3 (4)	3 (7)
Exposure			
Time of animal work per month (h/mo [range])	20 (1-174)	21 (1-144)	19 (1-174)
Mean exposure per month to:			
Rat allergens (eq. ng/m^3 * h/mo), GM ± 2* SD	89 (1-43,439)	85 (1-36,489)	105 (1-62,514)
Mouse allergens (eq. ng/m^3 * h/mo), GM ± 2* SD	173 (1-41,733)	148 (1-47,617)	223 (1-33,478)
Lung function			
Forced vital capacity (% predicted), mean ± 2* SD)	105 (83-129)	105 (84-127)	107 (83-132)
FEV_1 (% predicted), mean ± 2* SD	105 (80-129)	104 (81-127)	106 (79-133)
PD_{15} (μmol), GM (range)	16.4 (0.03-33.9)	19.9 (0.08-33.9)	12.2 (0.03-33.9)
Serology			
Atopy, no. (%)	43 (39)		
Total IgE (IU/mL), GM ± 2* SD	37 (3-465)	23 (2-227)	79 (12-536)
Nasal mucosa inflammation and permeability			
Albumin (μg/mL), GM ± 2* SD	6.3 (0.7-59.2)	6.4 (0.7-62.5)	6.2 (0.7-55.8)
α-2-Macroglobulin (ng/mL), GM ± 2* SD	163 (6-4,124)	158 (6-4,093)	171 (7-4,322)
Eosinophil cationic protein (pg/mL), GM ± 2* SD	628 (48-8,175)	641 (49-8,439)	606 (46-8,008)
IL-8 (pg/mL), GM ± 2* SD	103 (4-2,868)	143 (11-1,855)	62 (1-3,644)
Myeloperoxidase (ng/mL), GM ± 2* SD	37 (4-346)	44 (6-335)	27 (2-320)

GM, Geometric mean; *PD_{15}*, cumulative dose causing a 15% decrease in FEV_1.

discrimination). All reported *P* values were 2-tailed, and *P* values of less than .05 were considered significant.

RESULTS

Study population and occupational setting

Characteristics of the cohort are shown in Table I. At the start of the study, 4 subjects reported previous occupational sensitization to animals or plants. During the study, they had no contact with these allergens. As expected, atopic subjects had significantly higher levels of total IgE ($P < .001$) and tended to have a lower methacholine threshold ($P = .06$). At the start of the study, IL-8 and myeloperoxidase levels in the nasal lavage fluid were significantly higher in nonatopic subjects ($P = .020$ and $P = .033$, Table I).

Occurrence of occupational sensitization

Twenty-two (20%) participants had a new SLA, which was defined as a new positive skin prick test response (n = 21), a specific IgE level (n = 16), or both against rat or mouse urinary allergens. Ten participants became sensitized to rat urinary proteins, 4 to mouse urinary proteins, and 8 to both allergens. The incidence rate for sensitization in this study was 10.7 new cases per 100 person-years: 8.7 new cases per 100 person-years for rat-specific sensitization and 5.8 new cases per 100 person-years for mouse-specific sensitization. In sensitized employees with a positive RAST result, the mean skin test diameter was significantly larger than in employees with negative RAST results (last visit: 9 mm vs 3 mm, respectively; $P = .016$).

Baseline factors and development of occupational sensitization

The univariate odds ratios for development of occupational sensitization are shown in Table II. Because atopy was identified as a major risk factor in this study, with 42% of atopic participants having SLA, univariate odds ratios were calculated separately for this subgroup (Table II).

In addition to atopy, the number of parents reported to have allergic disease, total IgE level, bronchial methacholine threshold, working with rats, and estimated level of exposure to rats were associated with an increased risk of becoming sensitized to rats, as well as to mice. Except for methacholine threshold and number of parents reported to be atopic, these factors were also found to be associated with SLA in the subgroup of atopic subjects. Positive skin prick test responses and RAST results for common environmental allergens were also related to SLA (number of positive test results, as well as the level of specific IgE for house dust mite and grass pollen; results not shown). None of the parameters for inflammation and permeability in nasal lavage fluid showed an association with development of SLA.

When considering sensitization to rats or mice separately, the same parameters were found to be associated with the risk of sensitization (Table III).

TABLE II. Univariate odds ratios of baseline characteristics for development of occupational sensitization

	Whole cohort (n = 110)		Atopic subjects (n = 43)	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (y)	0.9 (0.8-1.0)	.126	0.9 (0.8-1.0)	.160
Sex	0.6 (0.2-1.6)	.341	0.7 (0.2-2.4)	.553
Data from questionnaires				
Smoking	1.2 (0.3-4.0)	.797	2.1 (0.4-10.8)	.377
One parent with allergies	3.7 (1.2-11.4)	.025	2.1 (0.5-8.3)	.275
Two parents with allergies	9.8 (2.1-45.9)	.004	10.4 (0.9-117.2)	.058
Pets at home	0.8 (0.3-2.0)	.633	0.9 (0.3-3.1)	.897
Job description				
Rat handlers	3.7 (1.4-10.0)	.009	10.5 (2.2-49.1)	.003
Mouse handlers	0.5 (0.2-1.2)	.110	0.3 (0.1-1.0)	.051
Rat and mouse handlers	0.5 (0.1-2.1)	.313	0.4 (0.1-2.2)	.295
Exposure				
Mean time animal work (h/mo)	2.4 (0.9-6.4)	.087	4.3 (1.0-18.4)	.052
Mean exposure per month to:				
Rat aeroallergens	1.6 (1.0-2.5)	.029	2.0 (1.1-3.5)	.017
Mouse aeroallergens	1.0 (0.7-1.5)	.981	1.0 (0.6-1.8)	.989
Lung function				
Forced vital capacity	1.0 (1.0-1.1)	.493	1.0 (1.0-1.1)	.729
FEV_1	1.0 (1.0-1.1)	.868	1.0 (1.0-1.1)	.964
PD_{15}	0.5 (0.3-1.0)	.036	0.4 (0.2-1.2)	.100
Serology				
Total IgE level	10.5 (3.1-35.5)	<.001	5.7 (1.1-28.9)	.035
Atopy	11.3 (3.5-36.8)	<.001		
Nasal mucosal inflammation and permeability				
Albumin	1.3 (0.5-3.4)	.580	1.1 (0.3-3.8)	.931
α-2-Macroglobulin	1.7 (0.8-3.3)	.139	1.4 (0.6-3.4)	.479
Eosinophil cationic protein	1.4 (0.6-3.1)	.452	1.0 (0.3-2.9)	.962
IL-8	0.9 (0.5-1.7)	.774	1.1 (0.6-2.3)	.731
Myeloperoxidase	1.0 (0.4-2.6)	.957	1.0 (0.3-3.1)	.987

PD15, Cumulative dose causing a 15% decrease in FEV_1; *OR*, odds ratio.

Cytokine production *in vitro* and SLA

Cytokine production by blood cells was compared between employees who became sensitized during follow-up and non-sensitized control subjects matched for atopic status. Nonstimulated and *Staphylococus aureus* Cowan I strain– or LPS-driven cytokine production in whole blood cultures is shown in Table E1 (available in this article's Online Repository at www.jacionline.org). No significant differences were found in cytokine production between patients and control subjects.

Multiple logistic regression

All parameters with a *P* value of less than .10 in the univariate analysis, except for exposure data, which are unavailable at the start of employment, were introduced in a multivariate logistic regression model to explain the development of SLA. The best model to predict SLA was determined by using a stepwise backward procedure (entry $P < .5$, removal $P > .10$; Table IV). In this model only atopy, total IgE level in serum, and number of allergic parents were retained as independent predictors for SLA. The presence of 1 allergic parent was associated with an increase in the odds of SLA by a factor of 4.8 (95% CI, 1.2–19.3). Atopy was in this model associated with an increase in the odds by a factor of 5.7 (95% CI, 1.5–22.2), and a 10-fold increase in total IgE level was associated with an increase in the odds by a factor of 8.2 (95% CI, 1.9–35.3). The model predicted 84.3% of the outcomes correctly. Furthermore, good agreement between predicted probabilities and the observed frequencies was reflected by a *P* value of .69 in the Hosmer-Lemeshow test.

Rat allergen exposure was an independent predictor in addition to atopy and total IgE level, but mean time of exposure and mouse allergen exposure did not contribute significantly.

In the validation cohort the predictive value of the number of allergic parents did not reach statistical significance in the multivariate model, but both atopy and total IgE level contributed significantly to the prediction of SLA (odds ratio for atopy, 6.3 [95% CI, 1.9–21.0]; odds ratio for total IgE level, 3.6 [95% CI, 1.6–8.0]); overall correct prediction by the model, 88.7%; Hosmer-Lemeshow $P = .77$). The ROC curve for the models including atopy and total serum IgE level are depicted in Fig E1 (available in this article's Online Repository at www.jacionline.org). The relationship between the predicted probabilities of development of SLA and the level of total IgE in atopic and nonatopic subjects for both cohorts is shown in Fig E2 (available in this article's Online Repository at www.jacionline.org). There is clustering of SLA in atopic employees with a high total IgE level. Choosing a cutoff value of 100 IU/mL for total IgE level limited the number of false-positive predictions to 10%. Based on these models, pre-employment counseling to advise against laboratory animal work in atopic applicants with a total IgE level of greater than 100 IU/mL might result in a 56% reduction of SLA in the group of atopic applicants, corresponding to a 45% reduction of SLA in the total group of applicants (Table V). In 7 (6%) participants fulfilling these criteria, sensitization did not occur during the 2 years of follow-up. Similarly, based on the data of the validation cohort, the reduction in sensitization in atopic subjects would have been 61%, corresponding to a 50% reduction in the

TABLE III. Statistically significant univariate odds ratios of baseline characteristics for development of sensitization to rats or mice

Risk factor	OR	95% CI	*P* value
Developing sensitization to rat allergens (n = 18)			
Atopy	11.67	3.12-43.63	<.001
House dust mite allergy (skin prick test)	5.16	1.78-15.00	.003
Grass pollen allergy (skin prick test)	7.00	2.33-21.00	.001
Total IgE level	9.26	2.65-32.33	<.001
Baseline PD_{15}	0.49	0.25-0.97	.04
Developing sensitization to mouse allergens (n = 12)			
Atopy	3.71	1.04-13.20	.043
Total IgE level	3.49	1.03-11.80	.045
Two allergic parents	17.67	2.65-117.62	.003

OR, Odds ratio; *PD15*, cumulative dose causing a 15% decrease in FEV_1.

whole cohort, at the expense of excluding 10% of the applicants from working with laboratory animals while they did not have SLA during a follow-up of 2 years. Therefore these data suggest that it might be possible to achieve a reduction of up to 45% to 50% in the occurrence of SLA with less than 10% false-positive prediction. Expressed according to the criteria put forward by Palmer et al,[7] a screening program aiming at excluding job applicants with atopy and a total IgE level of greater than 100 IU/mL would result in a number excluded to prevent 1 case of 2.6 (validation cohort, 3.7) and a number needed to screen to prevent 1 case of 16.7 (validation cohort, 21.9).

DISCUSSION

In this cohort the incidence of sensitization was very similar to the incidence of laboratory animal allergy reported in previous studies.[1-3] It is described in the literature that sensitization is frequently followed by the development of occupational allergy, which is usually defined as having work-related allergic symptoms in the presence of sensitization.[19] This was confirmed in our study. Symptoms of rhinoconjunctivitis were encountered most frequently (73%), and asthma symptoms were reported by 14% of the sensitized participants. According to the literature, approximately one third of all sensitized individuals might have allergic asthma.[1,4] We assume that the relatively short period of follow-up after sensitization might have underestimated the development of allergic asthma in our cohort.

The number of sensitized workers (n = 22) on which our predictive model is based is rather small, but the model was affirmed by the results obtained in a separate validation cohort. Inclusion of the validation cohort resulted in a total sample size of 270 LAWs comprising 44 workers who became sensitized. Nevertheless, sample size might have influenced the outcome, and additional studies should confirm the predictive value of the model.

The relatively low mean time of monthly exposure to animals and the low numbers of animal caretakers in our study does raise the possibility that our model might not be representative for full-time animal workers. It was previously suggested that atopy might be more of a risk factor for SLA with low levels of exposure.[3,6]

We found that the level of rat allergen exposure did contribute to the prediction of sensitization but did not affect the predictive value of atopy and total IgE level in multivariate analyses. Mean duration of exposure and levels of exposure to mice allergens did not contribute significantly. Because it is very difficult to make a reliable prediction of the level of exposure before appointment of employees, we considered a model including estimations of future exposure impractical for pre-employment counseling.

TABLE IV. Multivariate logistic regression

	Study cohort OR (95% CI)	Validation cohort OR (95% CI)
Best models to predict SLA		
Atopy	5.7 (1.5-22.2)	6.3 (1.9-21.0)
Log total IgE level	8.2 (1.9-35.3)	3.6 (1.6-8.0)
Allergic parents	4.8 (1.8-12.9)	Not included (NS)
Constant	0.001	0.006
Models including atopy and total IgE level		
Atopy	5.0 (1.3-18.3)	6.3 (1.9-21.0)
Log total IgE level	5.9 (1.7-21.0)	3.6 (1.6-8.0)
Constant	0.006	0.006

OR, Odds ratio.

In the facilities we studied, full-time animal caretakers represented only a minority of all employees with animal contact, and this group of employees showed little turnover. One of 6 animal caretakers (atopic and total IgE level of 138 IU/mL) became sensitized. Therefore it seems justified to focus on the larger group of employees with relatively low exposure in an attempt to reduce occupational sensitization.

We, like others,[2,3] identified atopy as a major risk factor for allergic sensitization. However, the total level of IgE did contribute significantly to a model that also included atopy. This was true for the whole cohort, as well as in the atopic and nonatopic subgroups. Therefore atopy and the level of total IgE appear to relate to 2 different aspects of allergic sensitization. Other factors associated with atopy, such as having a positive skin test response for house dust mite or pollen and a number of positive allergy test results, likewise showed positive associations with SLA but did not persist as independent predictors in multivariate models including atopy and total IgE level. These results were corroborated by retrospective analysis of the data from a previous cohort of animal laboratory workers.

Although the number of parents reported by the employee to have allergic symptoms was a significant independent predictor of SLA in our study, this was not found in the validation cohort in spite of having used similar questionnaires. We assume that the variable results of this and other subjective parameters are influenced by recall bias. Predictors based on questionnaires might therefore be less suitable for pre-employment counseling. The complementary predictive values of atopy and total IgE for SLA might be helpful in advising applicant LAWs about the risk of laboratory animal allergy. Because sensitization is rare in nonatopic individuals, counseling in this group does not seem appropriate. However, the group of atopic subjects is clearly at risk for SLA. As concluded earlier by others,[7,20] excluding all atopic subjects from laboratory animal work is not justified because this group might account for as much as 35% to 45% of the general population and sensitization occurs in less than 50%. However, identifying a group of atopic subjects with an increased risk of SLA based on a high level of total IgE might tip the balance in favor of pre-employment counseling. This study demonstrates that for atopic applicant employees, using a cutoff level

TABLE V. Cross-tables

	Study cohort*			Validation cohort*		
	Atopic	**Nonatopic**	**Total**	**Atopic**	**Nonatopic**	**Total**
Sensitization vs atopy, whole cohort						
SLA^+	18 (16%)	4 (4%)	22	18 (11%)	4 (3%)	22
SLA^-	25 (23%)	63 (57%)	88	39 (24%)	99 (62%)	138
Total	43	67	110	57	103	160
	Total IgE >100 IU/mL	**Total IgE <100 IU/mL**	**Total**	**Total IgE >100 IU/mL**	**Total IgE <100 IU/mL**	**Total**
Sensitization vs total IgE, atopic subjects only						
SLA^+	10 (23%)	8 (19%)	18	11 (19%)	7 (12%)	18
SLA^-	7 (16%)	18 (42%)	25	16 (28%)	23 (40%)	39
Total	17	26	43	27	30	57
	Atopic and IgE >100 IU/mL	**Nonatopic or IgE <100 IU/mL**	**Total**	**Atopic and IgE >100 IU/mL**	**Nonatopic or IgE < 100 IU/mL**	**Total**
Sensitization vs atopy in combination with total IgE, whole cohort						
SLA^+	10 (9%)	12 (11%)	22	11 (7%)	11 (7%)	22
SLA^-	7 (6%)	81 (74%)	88	16 (10%)	122 (76%)	138
Total	17	93	110	27	133	160

*Data are presented as numbers and percentages.

for total IgE of 100 IU/L, which was chosen to limit the number of false-positive results to 10%, will make it possible to achieve up to 50% reduction in the occurrence of SLA. Although up to 10% of the screened individuals who might be identified as being at risk will not have SLA during the initial 2 years of exposure, it is possible that this group might still have an increased risk of SLA after this period because occupational sensitization does occur after more than 2 years of exposure.[4,6] We realize that the models developed here might overestimate the predictive value, even though the results were affirmed in an independently recruited validation cohort. However, we believe that these results justify initiating prospective intervention studies to provide definitive proof of the value of pre-employment screening and counseling on the occurrence of occupational sensitization.

We measured *in vitro* cytokine production by blood cells at inclusion to investigate whether production of cytokines relevant for the balance between T_H1 and T_H2 lymphocytes was associated with the development of occupational sensitization. Previously, a reduced production of IL-12 and IFN-γ in patients with allergic asthma compared with that seen in nonatopic individuals was found.[21] Because SLA was mainly found in atopic subjects, control subjects were matched for atopy. Therefore in this study the results of these assays are primarily focused on differences within the group of atopic subjects and do not reflect differences that are expected to exist between nonatopic and atopic employees. The predictive value of an increased total IgE level is striking because cytokine production profiles that are assumed to control IgE production did not differ for most parameters. This apparent discrepancy might result from a larger degree of sampling error and a lower repeatability of cytokine production in the whole blood culture compared with detection of IgE in serum. It seems to indicate that total IgE level is a more reliable marker for the propensity of the immune system to mount an IgE antibody response.

In conclusion, in this prospective cohort study of starting LAWs, we found that atopy and the level of total IgE in serum were the main objective and independent predictors for occupational sensitization. These findings were corroborated by the results found in a previously recruited cohort. Based on our results, pre-employment counseling focused on applicant LAWs with atopy and a total IgE level of greater than 100 IU/mL might be able to significantly reduce the occurrence of occupational sensitization with an acceptable percentage of false-positive predictions.

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Clinical implications: The best model to predict the development of occupational sensitization includes atopic status and levels of total IgE. It might be able to reduce occupational sensitization by up to 50%.

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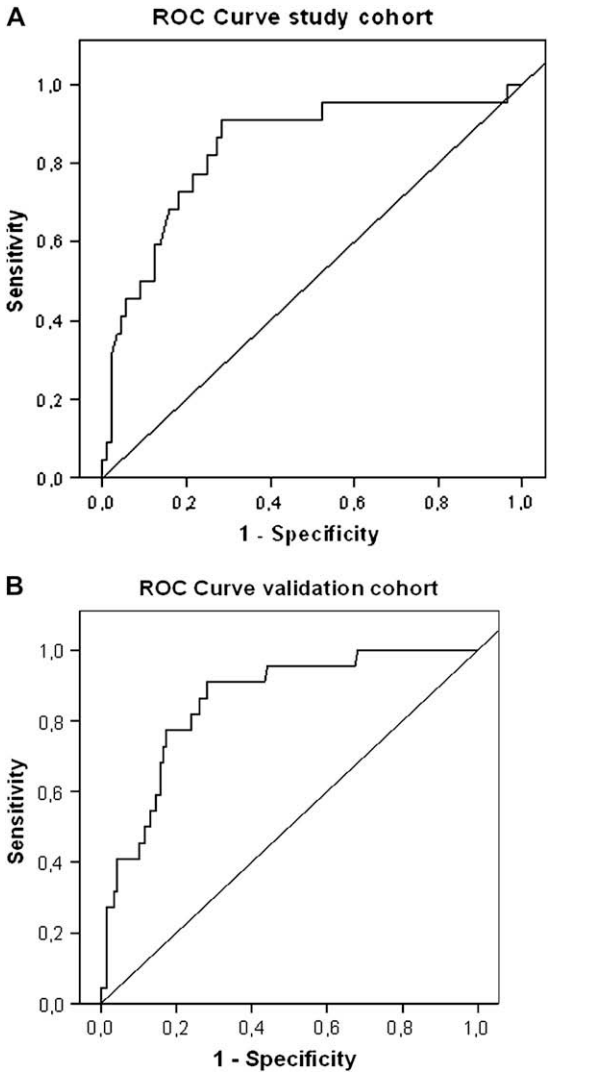

FIG E1. ROC curve of SLA as predicted by the combination of atopy and total IgE level. **A**, ROC curve in the study cohort: area, 0.837 (95% CI, 0.738-0.937). **B**, ROC in the validation cohort: area, 0.852 (95% CI, 0.776-0.928).

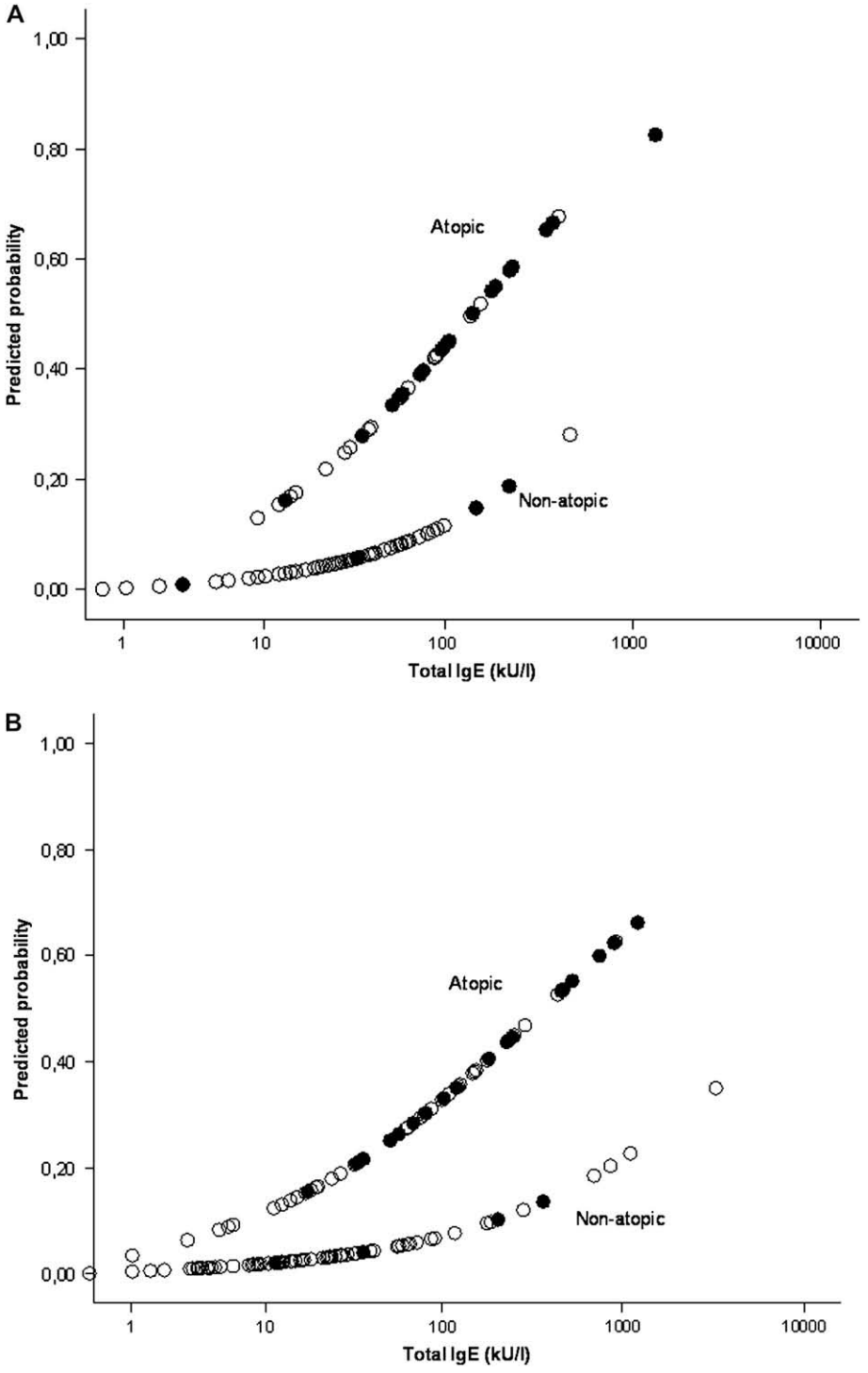

FIG E2. Probability of sensitization as predicted by the multivariate logistic regression model including atopy and total IgE level is plotted against level of total IgE. **A**, Study cohort. **B**, Validation cohort. *Open circles* indicate employees without sensitization, and *solid circles* indicate employees in whom SLA developed during follow-up. Sensitization is clustered in the atopic subjects with high total IgE levels.

TABLE E1. Cytokine production at baseline: Comparison between patients and control subjects

	All subjects		Atopic subjects	
	Control subjects	Patients	Control subjects	Patients
No.	22	22	18	18
Whole blood culture				
Nonstimulated assay				
IL-4 (pg/mL)	114 (92-141)*	87 (72-104)*	117 (92-149)*	90 (73-110)*
IL-6 (ng/mL)	12 (4-38)	5 (2-13)	14 (5-44)	6 (2-17)
IL-10 (pg/mL)	358 (253-507)	301 (219-413)	350 (250-489)	311 (223-434)
IL-12 (pg/mL)†	23 (16-34)	16 (11-22)	23 (15-35)	17 (11-24)
IFN-γ (pg/mL)	1,105 (801-1,524)*	733 (541-994)*	1,123 (796-1,585)	758 (530-1,083)
SAC-stimulated assay				
IL-4 (pg/mL)	135 (112-163)	132 (112-157)	136 (110-167)	130 (106-159)
IL-6 (ng/mL)	26 (15-45)	21 (10-41)	25 (14-45)	17 (7-37)
IL-10 (pg/mL)	258 (213-312)	242 (202-291)	245 (200-301)	240 (193-298)
IL-12 (pg/mL)†	158 (100-252)	139 (80-242)	170 (100-288)	124 (65-238)
IFN-γ (pg/mL)	2,934 (2,050-4,199)	2,221 (1,727-2,856)	2,965 (1,928-4,559)	2,084 (1,551-2,802)
LPS-stimulated assay				
IL-4 (pg/mL)	139 (115-169)	125 (106-149)	143 (115-178)	117 (98-140)
IL-6 (ng/mL)	53 (18-16)	48 (21-109)	65 (22-193)	47 (19-118)
IL-10 (pg/mL)	579 (400-839)	849 (586-1233)	633 (436-920)	889 (585-1352)
IL-12 (pg/mL)†	259 (163-412)	312 (162-600)	280 (164-475)	285 (147-552)
IFN-γ (pg/mL)	2,227 (1,216-4,108)	1,600 (1,214-2,107)	2,008 (1,103-3,656)	1,537 (1,134-2,083)

Results are expressed as geometric means (95% CIs).
SAC, *Staphylococcus aureus* Cowan I strain.
*$.05 < P < .10$
†Measured in stimulations in the presence of IFN-γ.