



Airway Inflammatory Responses Following Exposure to Occupational Agents

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Background: Airway inflammatory responses to specific inhalation challenges (SICs) with low-molecular-weight (LMW) and high-molecular-weight (HMW) agents have not been studied thoroughly. We assessed the changes in airway inflammatory cells following SIC in sensitized workers, and looked at the influence of various factors on the pattern of inflammatory responses to SIC.

Methods: Induced sputum analysis was performed in workers sensitized to LMW (n = 41) or HMW agents (n = 41) after a control day and after a positive SIC. Cell counts were compared with lung function and various clinical parameters.

Results: In the LMW group, eosinophils were increased following late asthmatic responses (median [interquartile range], $0.02 [0.04] \times 10^6$ cells/g vs $0.30 [0.80] \times 10^6$ cells/g and $1.0\% [3.5]$ vs $8.9\% [8.0]$, $P < .05$), as were neutrophil numbers ($0.8 [1.3] \times 10^6$ cells/g vs $2.3 [5.4] \times 10^6$ cells/g, $P = .04$). In the HMW group, eosinophil percentages increased both after early ($1.0\% [2.2]$ vs $5.5\% [14.5]$, $P = .003$) and dual asthmatic responses ($4.5\% [3.7]$ vs $15.0\% [13.7]$, $P = .02$). In the LMW group, the increases in neutrophils were higher in current smokers than in ex-smokers or nonsmokers. The length of exposure to the agent, tobacco use, and baseline percentage of eosinophils were independent predictors of the change in eosinophils, whereas age and baseline neutrophil percentage were predictors of the change in neutrophils.

Conclusions: This study confirms that eosinophils and neutrophils are increased after SIC, whatever the causal agent. The type of agent is not predictive of the inflammatory response to SIC. Smoking is associated with a more neutrophilic response after SIC with an LMW agent.

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Abbreviations: DAR = dual asthmatic reaction; EAR = early asthmatic reaction; HMW = high-molecular weight; ICS = inhaled corticosteroid; IQR = interquartile range; LAR = late asthmatic reaction; LMW = low-molecular weight; OA = occupational asthma; PC₂₀ = provocative dose of methacholine inducing a 20% fall in FEV₁; SIC = specific inhalation challenge

Occupational asthma (OA) is one of the most frequent occupational lung diseases, with a prevalence of up to 15% of adult asthma.^{1,2} It is induced by a substance encountered at the workplace to which the workers become sensitized.^{3,4} Substances causing

OA are classified into low-molecular-weight (LMW) agents (chemicals) or high-molecular-weight (HMW) agents (proteins).⁵ The latter act as common allergens through an IgE-mediated mechanism, whereas the mechanism of action is still being debated for LMW substances.⁶

The diagnosis of OA can be confirmed by performing specific inhalation challenges (SICs),⁷ involving exposing the subject to the suspected offending agent, or by performing serial peak-expiratory flow measures during periods at and away from work. In the majority of subjects experiencing a positive reaction,

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SICs induce a significant fall in expiratory flows after exposure to the sensitizer, but also an increase in sputum eosinophils.⁸

Consequently, sputum cell counts have been added to the routine investigation by SICs performed in the province of Quebec. However, SICs can induce various inflammatory reactions; other studies showed increases in eosinophils and/or neutrophils in the airways of subjects following an occupational challenge to various types of agents.⁹⁻¹² Lemi re et al¹³ showed that subjects with a noneosinophilic response to SIC had an increased decline in FEV₁ over time and did not show an improvement in airway responsiveness. We showed previously that LMW agents more frequently induce late asthmatic reactions (LARs) or dual asthmatic reactions (DARs), whereas HMW agents more often induce early asthmatic reactions (EARs).¹⁴ However, the inflammatory patterns associated with these different types of reactions have not been studied thoroughly.

Besides the offending occupational agent itself, many other factors can influence the type of airway inflammation induced by exposure to occupational agents. Although SICs should ideally be performed in subjects not treated with inhaled corticosteroids (ICSs) or any other asthma medication, in many patients this treatment cannot be interrupted without inducing an asthma exacerbation. Furthermore, the influence of active smoking on the airway inflammatory changes following occupational challenge is also unclear. In non-OA, smoking has been shown to induce an increase in sputum neutrophils.^{15,16}

Thus, more information is needed about the different inflammatory patterns of OA, and the factors influencing such responses. We herein present the results of an analysis of changes in airway inflammatory cells following laboratory exposure to HMW and LMW substances in sensitized workers and a comparison of those inflammatory responses with physiologic changes. We also aimed to investigate the influence of different factors on the airway inflammation observed after an occupational SIC.

MATERIALS AND METHODS

Subjects and Design

In this retrospective study, data obtained from the charts of subjects with OA confirmed with a positive SIC between 2001 and 2008 in two Canadian referral centers for OA (Institut universitaire de cardiologie et de pneumologie de Qu bec, Quebec City, and H pital du Sac -C ur, Montreal, Quebec) were analyzed. At that time, the investigation for OA included a first visit to collect baseline information, and subsequent visits for performing SIC. The SIC was performed in the laboratory and was used to confirm the diagnosis of OA. A methacholine challenge and an induced sputum were performed after the first day (control day; exposed

to a nonsensitizer) and 8 h after the end of exposure to the sensitizing agent, on the last day of the SIC. All methods remained the same throughout the study period. The current protocol was exempted from the need for approval by the Institut universitaire de cardiologie et de pneumologie de Qu bec and H pital Sac -C ur review boards because it met all requirements for use of anonymized data.

Subjects were aged 18 and over, had not experienced upper or lower respiratory tract infection within the 4 weeks prior to the SIC, had a stable respiratory condition, and produced induced sputum samples on the first and last challenge day. Charts were reviewed by trained research assistants. Data from the SIC, induced sputum cell counts, methacholine challenges, respiratory function tests, and medical history were analyzed.

Treatment with ICS was maintained during the investigation for patients taking these medications, but long-acting β_2 -agonists were stopped 72 h before the methacholine challenge and the SIC. Short-acting inhaled β_2 -agonists were withheld for at least 6 h before challenges.

Definitions

We defined an EAR to the SIC as a fall in FEV₁ of $\geq 20\%$ occurring within 1 h after the end of exposure to the sensitizing agent. An LAR was defined as a $> 15\%$ fall in FEV₁ occurring between 2 and 8 h after the end of exposure to the offending agent. A DAR was defined as the combination of an early and a late reaction.

Procedures

Spirometry and Metacholine Challenge: Spirometry was performed according to the recommendations of the American Thoracic Society.¹⁷ Methacholine challenge tests were done according to the tidal breathing method.¹⁸

Induced Sputum: Sputum was induced using the method described by Pin et al¹⁹ and modified by Pizzichini et al.²⁰ A differential cell count, including eosinophils, neutrophils, macrophages, lymphocytes, and bronchial cells, was performed.

Specific Inhalation Challenge: SICs were performed according to previously published methods.^{5,21} For each series of challenges, a control day was conducted, in which the subjects were initially exposed to a nonsensitizing agent (eg, lactose, solvents). During the SIC, FEV₁ was measured at baseline and every 10 min for the first hour, every 30 min for the second hour, and then hourly for at least 8 h after the end of exposure.⁵ On the following days, the time or dose of exposure to the occupational agent was increased progressively according to preestablished protocols for each type of substance. For agents causing OA through an IgE-mediated mechanism, such as HMW substances, exposure to increasing doses was performed progressively over 1 day, whereas the exposure for agents causing OA without a recognized IgE-mediated mechanism was completed over several days. The SIC was considered positive if an early, late, or dual reaction was observed.

Data Analysis

Data were expressed using mean $\pm$ SD or median (interquartile range [IQR]). Physiologic values were analyzed using a two-way analysis of variance model. Some variables were log transformed to stabilize variance. Cell data were analyzed using a four-factors mixed model: one factor (fixed) was associated with the comparison among the three groups of reactions; one was linked to the comparison between results from LMW agents and HMW agents (fixed factor); one was linked to the subjects (random factor); and one was associated with the comparison

among results at the two visits (fixed). This factor was analyzed as a repeated factor with interaction terms between the fixed factors and a compound symmetric structure for repeated measures. All cell count variables and percentages were log transformed to stabilize variance. Univariate and stepwise multivariate regression analyses were performed to determine the influence of extrinsic factors (baseline eosinophil or neutrophil percentage, age, maximal fall in FEV₁ after the SIC, length of exposure to the agent, sex, tobacco use, atopy, type of agent, ICS use, and type of reaction) on the change in eosinophils and neutrophils after the SIC. General additive models were used to investigate a relationship between the variables if it was expected to be of a complex form, not easily fitted by standard linear or nonlinear models. After investigation, the linear relationship was the appropriate functional form. The univariate normality assumptions were verified with the Shapiro-Wilk test. The multivariate normality assumptions were verified with the Shapiro-Wilk test after a Cholesky factorization. The Brown and Forsythe's variation of the Levene test statistic was used to verify the homogeneity of the variances. Pearson correlation coefficients were used to measure the relationship between the physiologic data and the change in cells. Results with *P* values ≤ .05 were considered significant. All analyses were conducted using the statistical package SAS, version 9.1.3 (SAS Institute Inc).

RESULTS

Subjects' Characteristics

The subjects' characteristics are summarized in Table 1. Among the 82 subjects evaluated, 41 were sensitized to LMW and 41 to HMW agents. LMW substances included mainly isocyanates, plicatic acid, and other chemicals, whereas the most prevalent HMW agents were flour and seafood. There was no difference in ICS use between the two groups (LMW, 63% vs HMW, 51%; *P* = .3). Following the SIC, the types of reaction were different between the two groups, with mostly LAR in the LMW group and EAR in the HMW group. The change in the provocative dose of methacholine inducing a 20% fall in FEV₁ (PC₂₀) was not statistically different between the two groups (mean change in PC₂₀ [double concentrations] ± SD: LMW, -1.27 ± 2.10; HMW, -0.62 ± 1.66; *P* = .2). However, a subanalysis in the LMW group showed that the increase in airway responsiveness (change in PC₂₀) was significantly greater in subjects sensitized to isocyanates (-2.43 ± 1.91) than in subjects sensitized to other LMW substances (-0.32 ± 1.69 double concentrations, *P* = .006).

Sputum Analysis Following SIC

In all subjects, total cell counts increased after the SIC (Table 2). Eosinophil percentages and numbers, as well as neutrophil numbers, were also increased after the SIC, although when comparing LMW with HMW agents, we found no differences between the two groups (Table 3). In the HMW group, a negative correlation (*r* = -0.41, *P* = .01) was found between

Table 1—Subjects' Characteristics

Characteristic	Type of Agent	
	LMW	HMW
No.	41	41
Age, y	40.5 ± 10.5	37.5 ± 9.7
Sex, No.		
Male	30	24
Female	11	17
Atopy, positive, ^a %	63	88
Smoking habit ^a		
Nonsmoker		
P-y	0 ± 0	0 ± 0
No. (%)	15 (37)	17 (41)
Smoker		
P-y	13.8 ± 15.5	13.1 ± 8.1
No. (%)	7 (17)	13 (32)
Ex-smoker		
P-y	11.5 ± 10.8	12.1 ± 6.4
No. (%)	17 (41)	11 (27)
Unknown		
P-y	NA	NA
No. (%)	2 (5)	0 (0)
Duration of asthma, y	4.5 ± 6.9	5.9 ± 9.9
Duration of exposure, y	10.0 ± 10.5	10.2 ± 8.7
Duration of exposure before onset of symptoms, y	6.1 ± 8.0	6.1 ± 7.5
Baseline FEV ₁		
L	3.3 ± 0.8	3.1 ± 0.9
% Predicted	97 ± 17	90 ± 17
Baseline FVC		
L	4.4 ± 1.0	4.1 ± 0.9
% Predicted	107 ± 15	102 ± 16
Baseline PC ₂₀	4.8 (0.2-128)	3.4 (0.04-128)
methacholine, geometric mean (range), mg/mL		
Type of reaction following SIC ^b		
Early, %	27	76
Late, %	60	8
Dual, %	13	16
Maximal FEV ₁ fall following SIC, %	24.4 ± 8.2	27.2 ± 9.8
Inhaled corticosteroid use, %	63	51

Data are presented as mean ± SD unless indicated otherwise. HMW = high-molecular weight; LMW = low-molecular weight; NA = not applicable; P-y = pack-years; PC₂₀ = provocative dose of methacholine inducing a 20% fall in FEV₁; SIC = specific inhalation challenge.

^a*P* < .05.

^b*P* < .001.

the change in eosinophil percentages and the duration of exposure before the onset of asthma symptoms. In the LMW group, the change in neutrophil numbers during the SIC was correlated with the number of pack-years subjects had smoked (*r* = 0.65, *P* < .0001). Furthermore, in the LMW group but not in the HMW group, the change in neutrophils after the SIC was greater in current smokers (median [IQR], 21.9% [28.1]) than in ex-smokers (3.6% [23.3]) and in nonsmokers (-2.0% [17.9]) (*P* = .02), whereas baseline neutrophil percentages were not different

Table 2—Induced Sputum Cell Counts Before and After Challenge

Parameter	Before SIC	After SIC	P Value
Total cell count, $\times 10^6/\text{g}$	1.9 (3.7)	3.3 (7.2)	.004
Eosinophils			
%	1.8 (4.0)	7.9 (14.9)	< .001
$\times 10^6/\text{g}$	0.03 (0.14)	0.27 (0.18)	< .001
Neutrophils			
%	41.8 (38.3)	45.5 (38.3)	.26
$\times 10^6/\text{g}$	0.7 (2.4)	1.6 (4.8)	.01
Macrophages			
%	43.2 (35.7)	33.9 (35.3)	.008
$\times 10^6/\text{g}$	0.9 (1.0)	1.1 (1.4)	.23
Lymphocytes			
%	1.3 (2.6)	1.0 (1.8)	.87
$\times 10^6/\text{g}$	0.03 (0.066)	0.03 (0.105)	.03

Data are presented as median (interquartile range). See Table 1 legend for expansion of abbreviations.

($P = .8$). In both groups, the changes in eosinophils and neutrophils were similar, regardless of ICS use (data not shown).

Sputum Analysis According to the Patterns of Reaction Following SIC

When analyzing the data according to the type of reaction following the SIC, total cell counts were increased in all subjects after EAR (median [IQR], 1.8 [4.4] vs 2.9 [7.1], $P = .046$). Eosinophil percentages were increased after all types of responses (EAR, 1.0% [2.4] vs 5.1% [13.7], $P = .007$; LAR, 1.8% [3.7] vs 8.2% [7.3], $P = .03$; DAR, 6.3% [2.8] vs 16.3% [16.0], $P = .01$), whereas eosinophil absolute numbers were significantly increased in early and late, but not in dual responses (data not shown).

In the LMW group, eosinophil numbers and percentages were increased following LAR ($0.02 [0.04] \times 10^6$

Table 3—Induced Sputum Cell Counts According to Offending Agent

Parameter	Before SIC	After SIC	P Value
Total cell count, $\times 10^6/\text{g}$			.84
LMW	1.9 (2.7)	3.8 (6.0)	
HMW	1.6 (4.8)	2.9 (7.6)	
Eosinophils, %			.10
LMW	2.5 (4.6)	8.8 (12.8)	
HMW	1.0 (2.5)	6.0 (15.0)	
Eosinophils, $\times 10^6/\text{g}$			.42
LMW	0.04 (0.14)	0.30 (0.60)	
HMW	0.03 (0.06)	0.27 (0.76)	
Neutrophils, %			.58
LMW	34.9 (37.6)	41.7 (44.2)	
HMW	52.2 (40.8)	47.0 (33.5)	
Neutrophils, $\times 10^6/\text{g}$			.92
LMW	0.7 (1.6)	1.4 (4.5)	
HMW	0.7 (3.4)	1.8 (5.0)	

Data are presented as median (interquartile range). See Table 1 legend for expansion of abbreviations.

cells/g vs $0.30 [0.80] \times 10^6$ cells/g and 1.0% [3.5] vs 8.9% [8.0], respectively, $P < .05$), as were neutrophil numbers ($0.8 [1.3] \times 10^6$ cells/g vs $2.3 [5.4] \times 10^6$ cells/g, $P = .04$). They were not significantly increased after EAR and DAR (data not shown). In the HMW group, eosinophil percentages were increased in both EAR (1.0% [2.2] vs 5.5% [14.5], $P = .003$) and DAR (4.5% [3.7] vs 15.0% [13.7], $P = .02$), whereas eosinophil numbers were increased after EAR ($0.03 [0.05] \times 10^6$ cells/g vs $0.26 [0.89] \times 10^6$ cells/g, $P = .04$). Eosinophils were not significantly increased after LAR in this group (data not shown).

Regression Analyses

Multivariate regression analyses showed that the presence of eosinophils at baseline, the number of pack-years, and a longer exposure to the offending agent resulted in a lower increase in eosinophils after the SIC (Table 4). Higher percentages of neutrophils at baseline resulted in a smaller increase of that cell type, whereas older subjects had a higher increase in neutrophils after the SIC (Table 5).

DISCUSSION

This retrospective study provides original information on inflammatory changes following exposure to workplace sensitizers, in relation to the pattern of change in expiratory flows following bronchoprovocation with the offending agent, in a large number of subjects. It confirms that airway eosinophils increase significantly following SIC, whatever the type of asthmatic response to the offending agent. Other studies^{22,23} showed that most workers with a positive SIC had higher sputum eosinophil counts when they were at work, whereas they decreased when they were removed from work. However, these studies did not specifically look at differences in the patterns of response according to the type of offending agent. The current study shows that sputum eosinophilia is common after exposure to both types of sensitizers and following all types of asthmatic responses. We also found increased neutrophil numbers after the SIC in all subjects, confirming the findings from other studies^{8,9,11,12} in smaller numbers of subjects. However, the contribution of neutrophils to physiologic changes or long-term outcomes remains uncertain.²⁴ Maghni et al²⁵ showed that inflammation persists many years after cessation of exposure to an occupational agent causing asthma and that the absence of remission could be related to the type or persistence of airway inflammation.

In the HMW group, we also observed a negative correlation between the increase in eosinophils and the duration of exposure to the agent before the onset of asthma symptoms, possibly explained by an

Table 4—Multivariate Analysis for Change in Eosinophil Percentages After SIC

Variable	Estimate (SE)	P Value
Intercept	18.09 (2.68)	< .001
Length of exposure	−0.33 (0.17)	.05
Tobacco use	−7.17 (2.80)	.01
Baseline eosinophil, %	−0.93 (0.23)	.001

$r^2 = 0.33$. See Table 1 legend for expansion of abbreviations.

“immunologic tolerance.”^{26,27} It may also indicate a shift toward a less eosinophilic type of asthma, as sometimes observed in obese subjects²⁸ and asthmatic smokers.¹⁶ Other contributing factors may include genetics,²⁹ the concentration of the inhaled agent,²¹ and continuous vs intermittent exposure.

In the LMW group, a correlation was found between the change in neutrophil numbers during the SIC and the number of pack-years subjects had smoked. Furthermore, current smokers had a higher increase in neutrophil percentages after SIC with an LMW agent than did ex-smokers and nonsmokers. These results suggest that current smoking could have influenced the neutrophilic response to the SIC. It has been demonstrated that cigarette smoke alters the airways and induces neutrophilia.^{15,16,30–33} LMW agents are also known to induce neutrophilia,⁹ and Di Franco et al³⁴ found a higher percentage of neutrophils in subjects exposed to LMW than to HMW agents, although it did not reach statistical significance. Thus, exposure to an LMW agent in a subject with an altered airway wall due to cigarette smoking seems to enhance neutrophil recruitment in the airways. The influence of smoking on the clinical outcome of patients with OA may possibly be detrimental; Maghni et al²⁵ showed that the presence of neutrophils after cessation of exposure was associated with a poorer prognosis. However, Lemi re et al¹³ found no association between smoking and airway obstruction or hyperresponsiveness in 19 subjects, 4 years after removal from their workplace.

We also found that in the LMW group, both eosinophils and neutrophils increased more markedly after LAR than EAR, whereas they increased after EAR and DAR with HMW agents. However, these results

Table 5—Multivariate Analysis for Change in Neutrophil Percentages After SIC

Variable	Estimate (SE)	P Value
Intercept	15.46 (14.36)	.33
Age	0.66 (0.28)	.02
Fall in FEV ₁ after SIC	−0.58 (0.37)	.13
Length of exposure	−0.49 (0.32)	.14
Nonimmediate reaction	9.57 (5.06)	.06
Baseline neutrophil, %	−0.54 (0.10)	< .001

$r^2 = 0.41$. See Table 1 legend for expansion of abbreviations.

were not confirmed after adjustments in the multivariate regression analysis, meaning we cannot conclude that the type of agent or response is predictive of the inflammatory response. The regression analysis showed that the baseline value of both cell types can help predict the magnitude of their increase after the SIC. The higher the levels of these cells at baseline, the lower the increase after the SIC. This could perhaps also be explained by an “immunologic tolerance,” as referred to here, although this remains to be studied further.

The main limitations of this study are its retrospective nature and the impossibility of withdrawing ICS treatment in some moderate to severe patients. Corticosteroids may affect the asthmatic response to the SIC and increase the length of exposure necessary to obtain a response, but that response still occurs and we have no evidence that its type would be changed by ICS.²¹ Moreover, there was no change in the pattern of treatment from the beginning to the end of the study period, so the effect of ICS was constant in a given patient.

This study on a large group of workers confirms that eosinophils and neutrophils are increased after SIC, whatever the causal agent. Eosinophils are also increased after all types of asthmatic responses. It also demonstrates that the type of agent is not predictive of the subsequent inflammatory response to the SIC. Active smoking, but not ICS intake, can influence the neutrophilic response to SIC with LMW agents and this should be taken into account in future studies that include smokers. It would be of interest to follow that cohort of workers in order to determine if the type of agent and the different patterns of responses, combined with the inflammatory responses, could help predict the outcome of subjects with OA.

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Dr Lemi re: contributed to the study protocol and writing of the manuscript.

Dr Dufour: contributed to the data collection, analysis, and writing of the manuscript.

Ms Chaboillez: contributed to the data collection, analysis, and writing of the manuscript.

Dr Boulet: contributed to the study protocol and writing of the manuscript, and as the guarantor of the manuscript.

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