# Is reactive airways dysfunction syndrome a variant of occupational asthma?

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**Background:** *Reactive airways dysfunction syndrome (RADS) or irritant-induced asthma is a syndrome that leaves subjects with asthma-like symptoms after one or more exposures to a high concentration of an irritant substance. The degree of reversibility of airway obstruction in subjects with RADS is nevertheless unknown, as is the degree of associated lesions at the airway level.*

**Methods:** *We compared the acute reversibility of forced expiratory volume in 1 second ($FEV_1$) after inhalation of albuterol (200 μg) in 15 subjects with RADS (12 cases caused by chlorine inhalation) with that of 30 subjects with occupational asthma (OA) caused by various agents. They were paired according to baseline airway obstruction (61% and 63% of predicted value in the RADS and OA groups), requirement for medication (bronchodilator only—7 of 15 subjects with RADS and 14 of 30 subjects with OA—as compared with bronchodilator + inhaled steroids in 8 of 15 subjects with RADS and 16 of 30 subjects with OA, respectively), and interval since removal from exposure (means of 30 and 24 months in the RADS and OA groups). In addition, five nonsmokers with RADS who had not received inhaled steroids underwent bronchoscopy with lavage and bronchial biopsies less than 2 years after the exposure.*

**Results:** *The percentage increase in $FEV_1$ over baseline after inhalation of albuterol was 10% ± 9% in the RADS group and 19% ± 16% in the OA group (p = 0.005). Only 2 of 15 subjects (13%) with RADS and 12 of 30 subjects (40%) with OA showed an improvement in $FEV_1$ of 20% or greater after inhalation of albuterol. Bronchoalveolar lavage showed an increased number of cells with a predominance of lymphocytes, and biopsy specimens showed increased basement membrane thickness in the five subjects with RADS who underwent bronchoscopy.*

**Conclusion:** *Subjects with RADS are generally left with less airway reversibility than those with OA. We suggest that this difference is secondary to distinct pathologic changes. (J ALLERGY CLIN IMMUNOL 1994;93:12-22.)*

***Key words:*** *Occupational exposure, occupational diseases, chlorine, bronchial diseases*

In 1985 Brooks et al.[1] described a new condition they called "Reactive airways dysfunction syndrome" (RADS), which consisted of persistent cough, shortness of breath, and wheezing after a single inhalational exposure to high concentrations of toxic products. This condition can also be referred to as "irritant-induced asthma"[2] and is characterized by the presence of nonspecific bronchial hyperresponsiveness in subjects who have no

*Abbreviations used*

| | |
|---|---|
| BAL: | Bronchoalveolar lavage |
| $FEV_1$: | Forced expiratory volume-one second |
| OA: | Occupational asthma |
| $PC_{20}$: | Concentration of methacholine causing a 20% fall in $FEV_1$ |
| RADS: | Reactive airways dysfunction syndrome |

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**TABLE I.** Baseline anthropometric, clinical, and functional results in subjects with RADS and OA with a latency period

| | RADS (n = 15) | OA with a latency period (n = 30) |
|---|---|---|
| Sex (M/F) | 14/1 | 23/7 |
| Age (yr) | 44 ± 14 | 50 ± 11 |
| Smokers and ex-smokers (*n*) (%) | 9 (60%) | 20 (66%) |
| Interval since end of exposure (mo) | 30 ± 19 | 23 ± 19 |
| Medication (BDT only/BDT + inhaled steroids) | 7/8 | 14/16 |
| $FEV_1$ (L) | 2.0 ± 0.6 | 2.2 ± 0.7 |
| $FEV_1$ (% pred)* | 61 ± 12 | 63 ± 14 |
| $FEV_1$/FVC (% pred)* | 82 ± 15 | 82 ± 15 |
| $PC_{20}$ (mg/ml) (mean, range)† | 2.0 (0.6-16) | 0.4 (0.05-1.6) |

Values are expressed as means ± SD unless stated otherwise.
*BDT*, Bronchodilator (inhaled $\beta_2$-adrenergic agent with or without theophylline derivative); *FVC*, forced vital capacity.
*See text for source of predicted values.
†Data available from 12 subjects (80%) with RADS and 22 subjects (73%) with OA with a latency period.

**TABLE II.** Baseline anthropometric, clinical, and functional results in the five subjects who underwent bronchoscopy

| Subject No. | Sex | Age (yr) | Intervals since last exposure (mo) | Smoking habits* | $FEV_1$ L | $FEV_1$ (% pred) | $FEV_1$/FVC (%) | $PC_{20}$ methacholine (mg/ml) |
|---|---|---|---|---|---|---|---|---|
| 1 | M | 36 | 24 | Ex-smoker (13 p-y; 9 yr) | 4.1 | 95 | 84 | 2.4 |
| 2 | M | 52 | 24 | Ex-smoker (6 p-y; 4 yr) | 3.6 | 100 | 80 | 1.2 |
| 3 | M | 35 | 26 | Ex-smoker (10 p-y; 6 mo) | 4.9 | 105 | 82 | 1.0 |
| 4 | M | 56 | 24 | Ex-smoker (20 p-y; 18 yr) | 3.0 | 92 | 89 | 7.0 |
| 5 | M | 36 | 30 | Non-smoker | 3.2 | 78 | 76 | 1.3 |

*FVC*, Forced vital capacity; *p-y*, pack-years.
*Pack-years and interval since cessation of smoking are in brackets; see text for sources of predicted values for $FEV_1$ and $FEV_1$/FVC.

previous history of asthma. Other publications have previously described a similar type of syndrome after toxic inhalation,[3-7] and case reports have recently been published.[8-10] Several agents can cause this syndrome including chlorine, ammonia, acid, fumes, and $SO_2$.

Kern[11] investigated 56 workers who had been exposed to high concentrations of glacial acetic acid. Approximately half of these subjects underwent a methacholine inhalation test 8 months after the exposure; the author found a dose-dependent relationship between the magnitude of exposure and the likelihood of the persistence of bronchial hyperresponsiveness. Kennedy et al.[12] also found that spirometric values were related to the number of acute chlorine gas exposures.

Because subjects with RADS are left with asthma-like symptoms and the presence of bronchial hyperresponsiveness, the assumption has been that RADS might reasonably be included in the definition of occupational asthma (OA). OA can be defined as a disease characterized by variable airflow limitation and/or airway hyperresponsiveness due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace.[2] This includes two main subgroups: (1) OA with a latency period, which is the most common type of OA,[13, 14] and (2) OA without a latency period (i.e., RADS). However, little is known about their similarity from a physiologic point of view. It is unknown whether the reversibility of

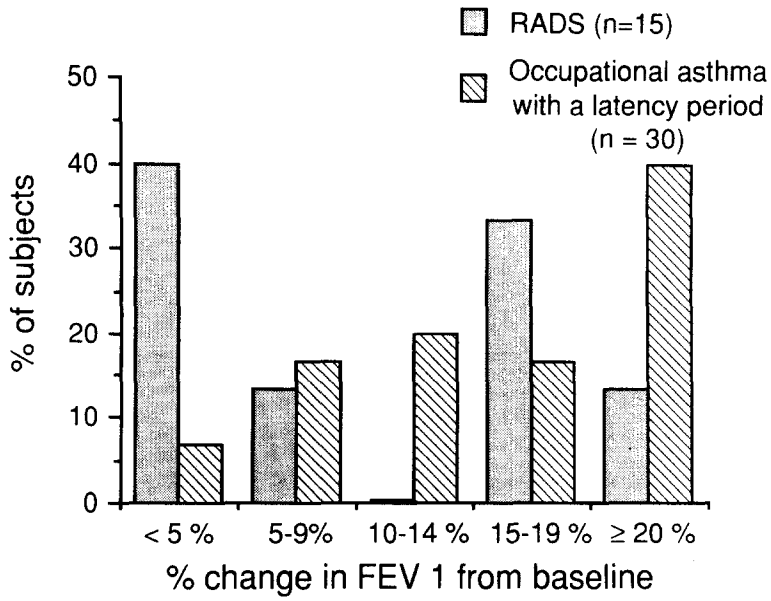


**FIG. 1.** Percentage of subjects with RADS and OA with a latency period according to the percent change in $FEV_1$ from baseline value after administration of 200 μg of albuterol.

**TABLE III.** Features of BAL in the five subjects with RADS who underwent bronchoscopy

| | BAL | | | | |
|---|---|---|---|---|---|
| Subject No. | Total number of cells ($10^6$) | Lymphocytes | Neutrophils | Eosinophils | CD4/CD8 |
| 1 | 88 | 49 | 1 | 0 | 1.9 |
| 2 | 32 | 5 | 2 | 0 | ND |
| 3 | 101 | 18 | 1 | 0 | ND |
| 4 | 33 | 25 | 1 | 0 | 1.7 |
| 5 | 22 | 14 | 0 | 1 | ND |
| Control subjects ($n$ = 10) | 21 ± 12 | 8 ± 4 | 1 ± 1 | 0.1 ± 0.3 | |

Mean ± SD values are given for normal control subjects.
*ND*, Not done.

airway obstruction, which is often present, is comparable in the two conditions. In their original report Brooks et al.[1] described the histologic features of two subjects who underwent bronchial biopsies; epithelial damage with nonspecific inflammation was observed. However, no eosinophils were found. Recently, Deschamps et al.[15] found a slight inflammatory response with spare lymphocytes and polymorphonuclear cells and no eosinophils. The same group of investigators also showed partial epithelial destruction and thickening of the subepithelial connective tissue by transmission electron microscopy.

The aims of this study were (1) to compare the reversibility of airway obstruction in subjects with RADS with that of subjects with OA with a latency period; and (2) to describe the pathologic changes in the bronchi of subjects with RADS. The hypothesis was that because of specific pathologic features, RADS can leave a functional state of airway obstruction that is less reversible than that of OA with a latency period.

## METHODS

### Subjects

For the reversibility study 15 subjects with a history of RADS who were referred to our asthma clinics for assessment of functional disability were asked to participate in a sequential order. Their baseline forced expiratory volume in 1 second ($FEV_1$) was less than 80% of the predicted value. Chlorine was the responsible agent in 12 instances and $NO_2$ in the three other

**FIG. 2.** Light micrograph of the bronchial biopsy specimen of a subject with RADS showing severe desquamation of epithelial cells *(arrows)*. Smooth muscle cells surrounded by reticulocollagenic fibrous tissue can be seen in the center part of the figure (Weigert-Masson trichrome stain; original magnification = ×150.)

subjects. All of these subjects had a history of acute symptoms (i.e., occurring within a few minutes or hours) after accidental exposure to these agents at work. They were all left with asthma-like symptoms. Subjects with RADS therefore fitted the definition of RADS proposed by Brooks et al.[1] Each subject with RADS was matched with two subjects with OA confirmed by specific inhalation challenges. The causal agents were isocyanates ($n$ = 13), flour and grains ($n$ = 8), soldering fumes ($n$ = 3), Western red cedar ($n$ = 2), psyllium ($n$ = 1), persulfate ($n$ = 1), rosin ($n$ = 1), and guar gum ($n$ = 1). The subjects were matched according to the following criteria: baseline airway obstruction ($FEV_1$ ± 10% predicted value), medication requirements (bronchodilator only as compared with bronchodilator + inhaled steroids), and interval since removal from exposure (±6 months). Information was available on the degree of responsiveness to inhaled methacholine (Wright nebulizer [Ferraris Medical Inc., Holland, N.Y.] output = 0.14 ml/min)[16] assessed by the provocation concentration causing a 20% fall in $FEV_1$ ($PC_{20}$) in 12 of the 15 subjects with RADS (80%) and in 22 of 30 subjects with OA with a latency period (73%). All subjects refrained from taking short-acting inhaled $\beta_2$-adrenergic agents for 8 hours before coming to the laboratory and from use of theophylline derivatives for 48 hours before being seen. Inhaled steroids were administered as usual. No subject took cromolyn or anticholinergic agents. Baseline anthropometric, clinical, and functional data are shown in Table I.

For the pathologic study, five subjects with RADS caused by chlorine also underwent bronchoscopy. The baseline anthropometric, clinical, and functional results for these subjects are shown in Table II. The criteria for selection were: (1) not currently a smoker, (2) no treatment with oral or inhaled steroids since the acute inhalation of chlorine, and (3) the presence of significant bronchial hyperresponsiveness.

Control subjects who had no present or previous history of smoking and denied respiratory symptoms were used for bronchoalveolar lavage (BAL) and biopsy comparisons.

## Functional assessments

Baseline spirometry was assessed according to the criteria of the American Thoracic Society[17] with a Collins 9L spirometer (Warren E. Collins, Inc., Braintree, Mass.). Albuterol (200 μg) was administered with a metered-dose inhaler under direct supervision of the technician. Spirometry was repeated 15 minutes later. Dose-response curves to inhaled methacholine were drawn on a noncumulative semilogarithmic scale, and $PC_{20}$ was interpolated on individual curves.

## Bronchoscopy and pathologic assessments

Airway inflammation was assessed by determining the cell differential from BAL and by light and electron microscopic studies of bronchial biopsy specimens.

Before bronchoscopy salbutamol and ipratropium bromide were given at doses of 200 and 80 μg, respectively, with metered-dose inhalers. Each subject received oxygen at a rate of 5 L/min by nasal catheter during the bronchoscopy. Vital signs, electrocardiographic readings, and oximetry were recorded throughout the procedure. Spirometry was done before and after the procedure, and patients were kept under observation for up to 1 hour after bronchoscopy. After local anesthesia of the throat, larynx, and bronchi was

**TABLE IV.** Features of the bronchial biopsy specimens from the five subjects with RADS who underwent bronchoscopy

| Subject No. | Total number of cells (X $10^{-3}$/mm²) | Neutrophils | Eosinophils | Mast cells | Lymphocytes | Plasmocytes | Monocytes/ macrophages |
|---|---|---|---|---|---|---|---|
| 1 | 24.7 | 2.4 | 3.0 | 0.6 | 16.6 | 0.3 | 0.9 |
| 2 | 16.3 | 1.5 | 2.3 | 0 | 10.5 | 0.7 | 0.7 |
| 3 | 13.6 | 1.0 | 1.8 | 0.2 | 9.0 | 0.2 | 0.5 |
| 4 | 17.2 | 0 | 0.2 | 0 | 14.3 | 1.4 | 0.5 |
| 5 | 11.2 | 0.4 | 0.2 | 0.2 | 8.4 | 0.6 | 0.8 |
| Control subjects ($n$ = 10) | 6.5 ± 0.7 | 0.3 ± 0.1 | 0.1 ± 0.1 | 0 ± 0 | 5.7 ± 0.6 | 0.2 ± 0.1 | 0.1 ± 0.1 |

Mean ± SD values are given for normal control subjects.

achieved with 2% and 4% lidocaine, the flexible bronchoscope (Olympus OES 10 fiberscope; Carsen Group, Inc., Markham, Ontario, Canada) was introduced into the bronchial tree and gently wedged into a segmental or subsegmental bronchus of the right middle lobe. Four 50 ml aliquots of normal saline solution (37° C) were instilled and aspirated with a syringe via the bronchoscope channel. After filtration, the fluids were kept on ice. The total number of cells was estimated on a hemocytometer. The cell differentials were obtained by counting at least 300 cells on glass cover preparations stained with Diff-Quik (Baxter Healthcare Corp., Scientific Div., McGaw Park, Ill.) or nonspecific esterase stains. The search for ciliated epithelial cells was done by counting 1000 cells on Diff-Quik–stained preparations. A metachromatic cell count was done with toluidine blue–stained preparations, and at least 1000 cells were counted.

Bronchial biopsies were performed immediately after lavage, on the same side. Six to eight specimens were taken from carinae of the right upper and lower lobes and segmental bronchi of the lower and upper lobes with conventional forceps. For light microscopy routine studies were carried out after fixation in Bouin's solution and samples were examined with a Leitz microscope (Wild Microscopes, Div. of Leica, Inc., Rockleigh, N.J.). The following light microscopic staining techniques were performed on all sections: hematoxylin and eosin, Masson trichrome, Giemsa, PAS, PAS with diastase digestion and Weigert's method.

All counts and measurements were made without knowledge of the source of the specimen. Measurements were derived from light micrographs (final magnification: ×250 for all measurements except basement membrane, which was ×2500), and included basement membrane thickness (mean of three measurements), percentage of epithelial desquamation, biopsy specimen surface in square millimeters and inflammatory cell type per square millimeter. Basement membrane thickness was measured from histologic sections stained with Masson trichrome. Percent cell desquamation was measured by evaluating the length of the basement membrane denuded of epithelial cells over the total length of the basement membrane. The biopsy specimen surface was evaluated for cell count as the surface of connective tissue excluding smooth muscle cells and mucous glands. Measurements were made with an image analysis system (Zeiss MOP 111, Carl Zeiss Inc., Thornwood, N.Y.). Samples for electron microscopic studies were fixed by immersion in Karnovsky's fluid, washed in cacodylate buffer, osmicated, dehydrated in alcohol, and embedded in Epon. Half of the samples were treated "en bloc" with uranyl acetate. All sections were stained with lead citrate and analyzed with a Jeol 100 CX electron microscope. Electron microscopic studies were used to evaluate cellular alterations, cilia abnormalities, and evidence of cell activation (degranulation) and basement membrane composition.

### Analysis of results

The reference values for spirometry were taken from the study by Knudson et al.[18] Increased bronchial responsiveness was considered to be present when $PC_{20}$ was less than or equal to 16 mg/ml.[19] Either parametric statistics (two-way analysis of variance, chi square distribution), whenever the distribution of data was normal, or Wilcoxon signed-rank test, for nonparametric statistics (when the distribution of data was not normal even after transforming data), comparing subjects with RADS with the paired groups of subjects with OA with a latency period (SYSTAT, Inc., Evanston, Ill.) were used for the statistical analysis of results. A $p$ value less than or equal to 0.05 was considered significant.

## RESULTS

### Functional assessment

There were no significant differences between the group of 15 subjects with RADS and the group of 30 with OA with a latency period according to the variables listed in Table I, except

| Nonidentified cells | Basement membrane thickness (mm) | Basement membrane thickness (μm) | Desquamation (%) |
|---|---|---|---|
| 0.9 | 17.5 | 24.5 | 57.3 |
| 0.3 | 21.3 | 29.8 | 39.2 |
| 0.7 | 21.0 | 29.4 | 38.8 |
| 0.7 | 20.9 | 29.3 | 41.7 |
| 0.6 | 15.9 | 22.3 | 62.0 |
| 0 ± 0 | 6.5 ± 0.4 | 9.1 ± 0.6 | 36.6 ± 3.7 |

for the degree of bronchial hyperresponsiveness, which was generally more pronounced in subjects with OA with a latency period. In 9 of 15 instances of RADS, one paired value of $PC_{20}$ was available, and in all instances the $PC_{20}$ value was larger in subjects with RADS. There were two subjects with RADS for whom two paired values of $PC_{20}$ were available for the subjects with OA with a latency period. In this instance values of $PC_{20}$ for the two subjects with RADS were in between those obtained for the two paired subjects with OA with a latency period.

The mean ± SD percent improvement over baseline was 9.6% ± 9.4% in the group with RADS and 19.4% ± 16.1% in the group with OA with a latency period. Because the distribution of data was not normal, the Wilcoxon signed-rank test was used to compare the individual results of the group with RADS to the mean results of each of the pairs in the group with OA with a latency period. The difference was highly significant ($p = 0.005$). The proportion of subjects whose $FEV_1$ improved after inhalation of albuterol is shown in Fig. 1. Six of the 15 subjects in the group with RADS (40%) had an improvement of less than 5% in $FEV_1$, whereas only 2 of 30 subjects in the group with OA (17%) did (chi square = 7.6, $p = 0.006$). Only two of the 15 subjects (16.7%) with RADS had an improvement in $FEV_1$ of 20% or greater, whereas this proportion was more than double (i.e., 12 of 30 [40%]) among the subjects with OA with a latency period (chi square = 3.3, $p = 0.06$).

### Pathologic findings

*BAL.* Features of the BAL fluid content and bronchial biopsy specimens from the five subjects who underwent bronchoscopies are shown in Table III. Although the number of subjects studied was not high enough for proper statistical comparison, it can be shown that subjects no. 1 and no. 3 had an absolute increase in the total number of cells recovered from BAL. Three of the five subjects showed an increase in lymphocytes in that values were higher than 2 standard deviations from the mean. The proportion of neutrophils and eosinophils did not differ from that of the control group. The CD4/CD8 ratio was normal in the two subjects in whom it was assessed.

*Light microscopic study.* Examination of the endobronchial biopsy specimens showed focal desquamation of the epithelial layer in association with squamous cell metaplasia (Fig. 2). The epithelial cells had evidence of cilia loss. The subepithelial connective tissue contained numerous inflammatory cells, mostly lymphocytes, and few polymorphonuclear neutrophils and eosinophils (Table IV). Mastocytes and monocytes/macrophages were occasionally observed.

The presence of reticulocollagenic fibrosis of the bronchial wall, as revealed by Masson trichrome stain, was striking (Figs. 3 and 4). The extent of fibrosis was moderate to severe. The reticulocollagenic fibers surrounded clusters of smooth muscle cells and nerve fibers. Elastic fibers were found, distributed in clusters, and were more numerous than usual, larger, and sometimes ruptured (elastosis). The basement membrane was severely thickened.

Dysplasia of the epithelial cells was observed in three cases and was characterized by large nuclei with dense granular chromatine granules and large nucleoli (Fig. 5).

*Electron microscopy.* Electron microscopy assessment revealed areas of squamous cell metaplasia, with large desmosomes associated with numerous tomofilaments (Fig. 6). Nuclear irregularities and nucleolar enlargement, characteristic of dysplasia, were striking. Persistent ciliated cells showed a significant reduction in the number of cilia, probably as a result of a poor genesis of basal corpuscles. In areas of epithelial denudation, we could observe the persistence of basal "reserve" cells still attached to the basement membrane by hemidesmosomes. In two cases osmiophilic inclusion bodies were seen in epithelial cells. They were round-shaped and made of degenerative granular material of an unknown nature.

## DISCUSSION

This study shows that the reversibility of airway obstruction after inhalation of a $\beta_2$-adrenergic

**FIG. 3.** High magnification of Fig. 1 showing loss of epithelial cells with thickening of basement membrane *(small arrow, top)*. The connective tissue shows numerous inflammatory cells, mostly lymphocytes. The *large arrow (bottom)* indicates packed elastic fibers, irregular in shape (original magnification ×300.)

**FIG. 4.** Light micrograph of bronchial biopsy specimen showing partial denudation of basement membranes *(B)*. Only a few basal cells remain attached to the epithelial border of the basement membrane. Numerous elastic fibers in connective tissue are indicated by the *arrow (bottom)*.

agent is more marked in subjects with OA with a latency period, which is the most common form of OA often occurring after sensitization on an immunologic basis, than in subjects with RADS. Very few case reports of RADS have described an acute response to an inhaled bronchodilator. Two of five subjects reported by Boulet et al.[20] with significant baseline bronchial obstruction showed a greater than 20% response to a bronchodilator. However, only three of the seven subjects (43%) reported by Härkönen et al.[6] had an improvement of more than 15% in $FEV_1$ after inhalation of a bronchodilator. The proportion of "responders" in terms of a 15% or greater increase in $FEV_1$ was similar (i.e., 7 of 15 [47%] in our study).

Our subjects all fitted the definition of RADS proposed by Brooks et al.[1] They did not have a history of long-term exposure in conjunction with the development of nonspecific respiratory symptoms compatible with chronic obstructive pulmonary disease. Five of the 10 subjects originally described by Brooks et al.[1] were also left with

**FIG. 5.** Light micrograph of bronchial biopsy specimen showing significant squamous cell metaplasia of the epithelium with dysplasia *(arrow)*. Large and dark nuclei on the luminal surface of the epithelium can be seen. The basement membrane is thickened (original magnification ×400.)

**FIG. 6.** Electron micrograph of bronchial biopsy specimen with epithelial cells devoid of cilia *(small arrows)*. The large arrow *(middle)* indicates an inclusion body with a dark osmiophilic center surrounded by a fine granular material (original magnification ×4500.)

airway obstruction ($FEV_1$ <80% predicted value), which seems to be a frequent finding in RADS.

Apart from the two subjects with RADS described by Brooks et al.[1] whose bronchial biopsy specimens showed features of nonspecific inflammation, pathologic information on RADS has only been described in two other reports as far as we know. Charan et al.[4] showed that two subjects who died as a result of acute inhalation of $SO_2$ had evidence of extensive sloughing of the mucosa of the large and small airways along with hemorrhagic alveolar edema; the latter probably explains their deaths.[4] Brooks et al.[1] described desquamation of the respiratory epithelium in one of their two subjects. They showed that the inflammatory infiltrate primarily consisted of lymphocytes and plasma cells. Deschamps et al.[15] showed partial epithelial destruction and thickening of the subepithelial connective tissue. This

was also documented in our subjects. No information on BAL has been reported. Our study shows an increase in the number of cells in BAL with a predominance of lymphocytes in three of the five subjects. Furthermore, the microscopic picture revealed subepithelial thickening with fibrosis, a finding not reported before. These fibrotic changes might offer some explanation for the lesser reversibility in airway obstruction after bronchodilator administration. Finally, electron microscopy showed deciliated cells with the presence of inclusion bodies. The exact nature of these bodies is still undetermined. Further pathologic studies with immunohistochemical staining would be required to define more clearly the precise nature and activation of the cell infiltrate, as well as the nature of collagen.

The aim of this study was to compare the reversibility in airway obstruction after inhalation of a $\beta_2$-adrenergic agent in subjects with RADS and subjects with OA with a latency period. Because there were differences, we hypothesized that this might be explained by distinct pathologic features in the subjects with RADS. Our pathologic data are descriptive, and we did not attempt direct comparisons of subjects with either occupational or nonoccupational asthma. This would have required a larger number of subjects, making statistical comparisons possible. This being said, it is worth mentioning that some of the pathologic features reported in our study are different from what is generally seen in asthma and OA. Although mucosal desquamation seems to be a key feature of asthma[21, 22] and OA,[23] the extent of basement membrane thickness seems unique to RADS. Paggiaro et al.[24] first noticed increased thickness of basement membrane in OA, although the magnitude was not assessed. To determine whether there are any correlations between basement membrane thickness and responsiveness to methacholine, we recently examined data obtained in our center (Boulet et al., in preparation), which show the thickness of basement membrane in different groups of subjects, including those with allergic rhinitis, as recently described.[25] Thickness of the basement membrane (in microns) expressed as mean values are as follows: chronic cough ($n$ = 19), 7.6; allergic rhinitis ($n$ = 11), 9.1; allergic asthma ($n$ = 6), 9.9; OA (high molecular weight agents, $n$ = 11), 16.7; OA (low molecular weight agents, $n$ = 7), 17.2; RADS ($n$ = 5), 27.1; and normal control subjects ($n$ = 10), 6.5. These figures are to be compared with a mean thickness of 8.7 μm in nine subjects with OA caused by toluene diisocyanate in the study by Saetta et al.[23] It was impossible in our subjects with RADS to distinguish the true from the reticular portions of the basement membrane, as in the study by Saetta et al.,[23] because of the rearrangement of the basement membrane. An increase in eosinophils was not found in our subjects with RADS. This is generally a feature in asthma.[26] Even if four of our five subjects were ex-smokers, all of them except one had stopped smoking for more than 1 year, and the features described in BAL fluid and biopsy specimens are not those generally encountered in smokers or ex-smokers. The findings of our study would obviously need to be extended to a larger number of subjects with RADS and compared with a sample of subjects with OA not receiving antiinflammatory preparations after they were removed from the offending agent.

It is also relevant to mention that bronchoscopy was carried out 1 to 2 years after the acute episode. This represents the chronic stage of the condition, and is likely that the picture would be different if the bronchoscopy had been done at an earlier stage. However, it is important to stress that these changes represent the "natural history" of the condition because our subjects did not receive oral or inhaled steroids and were either nonsmokers or had only a mild smoking history.

Subjects who were assessed by bronchoscopy all had normal airway caliber and demonstrated only mild bronchial hyperresponsiveness. It is interesting to note that even with what could be interpreted as minimal functional changes, most of our subjects demonstrated extensive pathologic changes. Further assessments of more severe cases of RADS would be interesting. However, such studies, if performed in the chronic stage of the resolution process, would be difficult because steroids are frequently administered in the early stage of the disease. This could obviously modify the histologic features. Such studies would be interesting in the first week after acute inhalation and serially thereafter because there is reason to believe that the worst functional effect occurs in the first week after the inhalational exposure, with improvement over time.[5]

The reversibility of airway obstruction was assessed in an acute way after administration of a $\beta_2$-adrenergic agent. It would be interesting to do the same assessment after administration of ipratropium bromide or a treatment with oral and/or

inhaled steroids, particularly in the short interval after the acute inhalational exposure. Even if steroids are frequently used immediately after such episodes (which was the case in many of our subjects, although the frequency of this could not be obtained with precision because the information was retrospective), it would be important to know what their effect is, if any, from a functional and pathologic point of view. Murphy et al.[3] describe no benefit in terms of $FEV_1$ in one subject who received steroids orally for 4 months. However, it is difficult to determine whether this subject who inhaled cleansing agent fumes had what could be referred to as RADS, because symptoms appeared 6 to 8 weeks after exposure.

We conclude that subjects with RADS assessed in the chronic stage of their condition do not seem to generally show reversibility of airway obstruction with an inhaled $\beta_2$-adrenergic agent to the same extent as subjects with OA with a latency period do. This lesser reversibility can be related to a different pathologic picture in RADS than is normally observed in OA and asthma. It is tempting to say that RADS may well represent a variant of OA that does not share the features of OA with a latency period. However, this still remains hypothesis that warrants confirmation. Further assessment of the reversibility after administration of other bronchodilators and antiinflammatory preparations are used in the acute and chronic stages of the condition is required. It would also be interesting to relate these functional findings to the pathologic picture. This information is needed because RADS is becoming a more commonly recognized condition. Further characterization of its natural history and acute and chronic physiologic and pathologic features needs to be done.

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# Fine specificity of B-cell epitopes on *Felis domesticus* allergen I (*Fel d* I): Effect of reduction and alkylation or deglycosylation on *Fel d* I structure and antibody binding

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*The repertoire of B-cell epitopes on the major cat allergen,* Fel d *I, was analyzed with monoclonal antibodies (MoAbs) in topographic mapping studies and in immunoassays with antigen derived from other cat* (Felidae) *species. Four essentially nonoverlapping epitopes on* Fel d *I, designated Fd1A to D, were defined by use of 15 anti* Fel d *I MoAbs in cross-inhibition radioimmunoassay. Only MoAbs directed against epitope Fd1B bound to putative* Fel d *I homologues in hair and dander extracts from seven other feline species (*Panthera *species, [*n = *5],* Leptailurus serval, *and* Leopardus pardalus*). Quantitative monosaccharide analysis showed that* Fel d *I was a glycoprotein, containing high levels of fucose, as well as glucosamine, galactose, and mannose. Binding of MoAbs and human IgG or IgE antibody to native, reduced and alkylated or deglycosylated* Fel d *I was compared by means of immunoprecipitation and immunoassay, and the effects of these treatments on the structure of* Fel d *I were analyzed by sodium dodecylsulfate–polyacrylamide gel electrophoresis. On reduction and alkylation,* Fel d *I dissociated into 14 kd and 3.2 kd peptides, and deglycosylation with trifluoromethane sulfonic acid produced a 12 to 14 kd peptide. These procedures resulted in a 100- to 1000-fold loss in murine or human antibody binding activity and caused significant loss of secondary structure, as judged by circular dichroism spectroscopy. Treatment with potassium hydroxide also caused a marked loss in antigenic reactivity. In contrast, enzymatic deglycosylation generated a 9 kd peptide, which showed strong reactivity with murine and human antibodies, comparable to native* Fel d *I. The results show that MoAbs define a broad repertoire of B-cell epitopes on* Fel d *I, one of which is expressed by other cat species. These epitopes are conformational and do not appear to involve oligosaccharide residues. (J ALLERGY CLIN IMMUNOL 1994;93:22-33.)*

***Key words:*** *Cat allergen, epitopes, monoclonal antibodies, glycoproteins, asthma*

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