

Occupational allergic rhinoconjunctivitis and asthma to goat and cross-reactivity with cow epithelium

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Background: The development of sensitization and symptoms after the inhalation of epithelial allergens is common.

Objectives: To investigate the allergic response (in vivo and in vitro) of 3 individuals clinically sensitive and occupationally exposed to cow and goat and to evaluate the allergenic cross-reactivity between cow and goat epithelium extracts.

Methods: Three patients—a butcher and 2 doctors in veterinary medicine—were evaluated. These patients reported allergic respiratory symptoms after occupational exposure to goats and cows. Extracts were prepared from epithelia of both animals. All the patients underwent organ-specific allergen challenges with cow and goat extracts. Four nasal and 2 bronchial challenges were conducted following standardized procedures.

Results: All 3 patients had positive challenge results with the offending allergen extract. Specific IgE to goat allergens was detected in all patients. Several IgE-binding bands were detected. Inhibition assays (enzyme allergosorbent test and immunoblots) confirmed moderate-to-high cross-reactivity between goat and cow extracts.

Conclusions: We confirm clinical sensitivity and specific IgE binding to goat and cow allergens in occupationally exposed individuals. There was good correlation among the clinical history, exposure, and the laboratory findings.

Ann Allergy Asthma Immunol. 2006;96:579–585.

INTRODUCTION

Epithelial allergens are common causes of acute and chronic allergic respiratory diseases. The most important epithelial allergens are derived from cats, dogs, horses, and cows, and allergic sensitization may occur at home or at the workplace.^{1,2} Several allergens have been characterized using biochemical and immunochemical techniques.³ The major allergen of bovine dander, Bos d 2, has been sequenced and cloned.⁴ Sheep, cows, and goats have economic importance in Mediterranean countries for domestic and commercial purposes. Humans may come in contact with these animals for different reasons. Doctors in veterinary medicine, butchers, and shepherds may encounter mammalian allergens owing to their professional activities.

Although allergy to goat's and cow's milk has been widely studied,^{5,6} allergy to epithelium, especially goat epithelium, has not been analyzed, particularly the allergenic composition of dander and its clinical implications. Goats belong to the order Artiodactyla, suborder Ruminantia, family Bovidae (which includes antelopes, gazelles, cattle, sheep, goats, and relatives), and subfamily Caprinae (sheep, goats, serows, and chamois)⁷ and were the earliest domesticated animals. The

most common domestic species in Spain is *Capra hircus*, originally native to Asia.

Cross-reactivity among deer, cow, horse, and goat allergen extracts has been described.^{8,9} In Poland and other European countries, sensitization to roe deer (*Capreolus capreolus*) has been described.¹⁰ In this study, patients were not exposed to goat but had specific IgE to goat and cow, possibly due to cross-reactivity among roe deer, goat, and cow allergens. This cross-reactivity could be due to the presence of lipocalins (20–25 kDa) in these extracts.¹¹ The aims of this study were to investigate the allergenicity of goat and cow dander, in vivo and in vitro, in 3 patients occupationally exposed to these 2 animals and to evaluate the allergenic cross-reactivity between both epithelia.

MATERIALS AND METHODS

Patients

All the patients were informed of the nature and risks of the study and signed the appropriate informed consent forms before entering the study. The study was approved by the ethics committee of Hospital de la Vega Baja, Orihuela, Alicante.

Patient 1. A 30-year-old male butcher who has worked in a slaughterhouse for 6 years reported previous sneezing and nasal blockage in the spring, associated with asthma when the symptoms are more intense. In the past 2 years he has had pruritus associated with urticaria after contact with calves, dogs, and cats. In the past year he experienced rhinitis and asthma each time he removed the skin of a calf. In these cases, he had to stop working and use bronchodilator therapy.

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Received for publication April 12, 2005.

Accepted for publication in revised form September 28, 2005.

He also reported rhinitis symptoms when he cut up goats, lambs, and horses. He tolerated ingestion of the meats of these animals. He received symptomatic treatment (antihistamines, bronchodilators, and inhaled corticosteroids) to control symptoms.

Patient 2. A 34-year-old male professor of veterinary medicine has exhibited rhinoconjunctivitis for the past 6 years when exposed to goats, sheep, and cows. He does not have symptoms when in contact with dogs, cats, horses, chickens, and rabbits. He supervises a goat farm and experiences these symptoms when in the proximity of the farm. He also experiences rhinoconjunctivitis symptoms when in the presence of freshly milked nonpasteurized goat's milk. He tolerates the ingestion of cooked meat and milk. He experiences rhinoconjunctivitis and, occasionally, asthma when in contact with cows.

Patient 3. A 27-year-old female veterinary doctor has experienced symptoms of rhinoconjunctivitis for the past 5 years when in contact with cows, goats, and horses. She also has contact with pigs and dogs, without symptoms.

Allergen Extracts

One hundred grams of cow and goat hair were purchased from Isoquimen, Harlan Iberica, Barcelona, Spain. The backs of live animals younger than 1 year were shaved using an electric shaver. The hair samples were stored in sealed plastic bags and shipped to the laboratory together with a certificate of analysis in which a doctor in veterinary medicine stated that the samples were collected from healthy animals free of infectious diseases and infectious agents. The samples were collected from herds in which no bovine spongiform encephalopathy had been declared previously. Preparation of the extracts complied with the guidelines contained in the *European Pharmacopoeia*¹² to minimize the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products.

The hair samples were incubated in acetone (1:40 wt/vol) for 16 hours, and then the dander was separated by sequential sieving through a stainless steel sieve with a 1-cm mesh and a Whatman No. 1 filter (Whatman PLC, Middlesex, England). The dander collected on the filters was air-dried. Afterward, cow and goat dander were individually extracted, 1:40 wt/vol, in 0.01M phosphate-buffered saline (PBS) (pH 7.4) for 16 hours at 4°C, centrifuged at 16,000g for 30 minutes, dialyzed against bidistilled water, sterile filtered, frozen, and freeze-dried. The protein content of the extract was measured using the Lowry-Biuret method (Sigma-Aldrich Corp, St Louis, MO).¹³ The cow and goat extracts contained 559 and 653 µg of protein per milligram of freeze-dried material, respectively. Skin prick tests were prepared with cow and goat extracts at a final concentration of 5 mg/mL of freeze-dried material.

Skin Tests

All 3 patients underwent skin testing with a battery of standardized aeroallergens, including *Dermatophagoides* species,

Olea europaea, *Cupressus* species, *Platanus acerifolia*, mixed grasses, *Salsola kali*, *Parietaria judaica*, *Artemisia vulgaris*, *Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium notatum*, cockroaches, dog and cat dander, and feathers (Laboratorios LETI, S.L.). All skin tests were performed on the volar surface of the arm following the recommendations of the European Academy of Allergology and Clinical Immunology.¹⁴ The cow and goat extracts were tested in duplicate at 4 dilutions: 0.005, 0.05, 0.5, and 5 mg/mL. These same tests were conducted in 6 control patients who were regularly exposed to cows and goats but who did not experience allergic respiratory symptoms related to this exposure.

Nasal and Bronchial Provocation Tests

The challenges were conducted in a hospital facility fully equipped to treat an acute exacerbation of respiratory disease. The tests were conducted by trained personnel, and immediate access to medical assistance was always available.

Nasal provocations. A nasal provocation test with goat extract was conducted in all 3 patients and with cow extract in 1 patient. Before conducting the nasal challenges, skin tests were performed in duplicate in both forearms with 10-fold increasing concentrations of the test allergens, starting at 0.005 mg/mL, until 5 mg/mL was reached. The diameters of the wheals were measured, and the concentrations that elicited a wheal size of 3 mm were selected to start the nasal provocation test. Nasal corticosteroids and antihistamines were withdrawn 3 weeks and 3 days before the start of the study, respectively. Patients were advised to avoid any exposure to animals for at least 2 days before challenge testing. Before starting the nasal challenges, patients waited 30 minutes to give the nasal mucosa time to acclimatize to local conditions. Nasal responsiveness was monitored by the number of sneezes. Specific nasal provocation tests were performed by means of anterior active rhinomanometry using a rhinomanometer (Datospir-164; Sibelmed, Barcelona, Spain). Nasal resistance was expressed as pascal seconds per cubic centimeter. The increase in resistance was calculated to determine the positivity of the test. First, rhinomanometry was conducted to determine the basal level of nasal resistance of the nose. Afterward, 0.1 mL of PBS was instilled in the nostril to determine the nasal hyperreactivity for diluents. If rhinomanometry showed a significant increase in nasal resistance, the test was not continued.

Nasal challenges were performed with increasing doses of the extract at 15-minute intervals after a sham challenge with PBS. Extracts were sprayed into each nostril by means of a nasal pump spray delivering a fixed dose of 0.1 mL of solution. The nasal response was measured 10 minutes after each challenge. An increase in nasal resistance greater than 100% over the control solution (PBS) was considered a positive reaction, and the nasal provocation test was stopped.^{15,16} In the event that the administered dose did not produce a positive reaction, a 10-fold higher dose was administered until the highest dose of 5 mg/mL was reached. The same challenge test (at 5 mg/mL) was performed on 3

nonsensitized control patients, obtaining negative results in all cases.

Bronchial provocations. The recommendations outlined by Sterk et al¹⁷ were followed to ensure the safety of the procedure. The use of all medications, which could interfere with the results of the challenges, was stopped before the tests. The patient clinical condition was stable at the time of the challenge. Baseline spirometry was performed to confirm that the forced expiratory volume in 1 second (FEV₁) was greater than 70% of predicted. Nonspecific bronchial challenge tests were performed with methacholine according to the method of Cockcroft et al,¹⁸ with some modifications.¹⁹ Allergen-specific bronchial challenge was conducted with a lyophilized cow extract reconstituted with buffer. The aerosolized particles were generated by a pressurized nebulizer (Hudson 1720; Hudson, Temecula, CA), with an input of 7 L/min and an output of 0.17 mL/min.

The patient underwent a control challenge with buffer at tidal breathing for 2 minutes before antigen provocation, and the FEV₁ was recorded. Allergen dose was increased by 10-fold increments at 60-minute intervals, and FEV₁ was measured 20 minutes after the inhalation of each concentration. The test was discontinued when there was a decline in FEV₁ of 20% or more with respect to the buffer control value. The test result is expressed as the provocative concentration of allergen that caused a decrease in FEV₁ of 20% (PC₂₀), as estimated by interpolation of the last 2 concentrations. At the end of inhalation testing, peak expiratory flow was monitored hourly during the day until bedtime.²⁰ The same bronchial challenge test was performed in 2 nonsensitized control subjects. In both cases, the results were negative at a maximal allergen dose of 5 mg/mL.

Specific IgE Determinations by Enzyme Allergosorbent Test and Inhibition Assays

Specific IgE to cow and goat epithelia was determined in serum samples from the 3 patients using the Pharmacia CAP System (Pharmacia, Uppsala, Sweden). Extracts were coupled to cyanogen bromide-activated paper disks at a protein concentration of 1 mg/mL.²¹ Serum samples were diluted 1:5 in PBS containing 1% bovine serum albumin and 0.1% Tween 20 and incubated overnight at 4°C. After 3 washes in PBS-0.1% Tween 20, the disks were incubated overnight in a 1:10,000 dilution of biotinylated rabbit antiserum to human IgE (DAKO A/S, Copenhagen, Denmark) supplemented with 5% normal rabbit serum. After further washing, biotinylated

antibodies were detected with streptavidin-peroxidase (250 ng/mL) and a mixture of ortho-phenylene-diamine at 3 mg/mL and hydrogen peroxide (all from Sigma-Aldrich Corp) and read at 492 nm. For the inhibition assays, individual serum samples were incubated in varying amounts of the inhibitor (from 100 to 0.1 µg) for 2 hours at 37°C, followed by the procedure described previously herein for specific IgE detection. Results are expressed as percentage of inhibition.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Immunoblotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described.²² Extracts were analyzed on 15% acrylamide discontinuous gels with 4% acrylamide stacking gels. A total of 100 µg of freeze-dried material was loaded on the gels. After electrophoresis, gels were Coomassie stained and analyzed. A total of 150 µg/cm was resolved by SDS-PAGE and blotted onto nitrocellulose membranes (Sigma-Aldrich Corp) in Towbin buffer²³ with 20% methanol in a Hoeffer apparatus (Amersham Biosciences, Uppsala). Blotted membranes were blocked for 1 hour in Tris-buffered saline (pH 7.4) (Tris hydrochloride, 50 mmol/L, and sodium chloride, 150 mmol/L) containing 5% skimmed dry milk. After blocking, 3-mm strips were cut and incubated overnight with serum samples, diluted 1:5 in incubation buffer (blocking solution supplemented with 0.1% Tween 20). The next day, the membranes were washed, and bound IgE was detected using a biotinylated mouse monoclonal antibody to human IgE diluted 1:1,000 (Ingenasa, Madrid, Spain), followed by streptavidin-peroxidase (Sigma-Aldrich Corp). Detection was performed using the ECL system (Amersham Biosciences, Uppsala).

Immunoblot inhibition experiments were conducted by inhibiting IgE binding to goat and cow extracts. Inhibition was performed as follows: 200 µL of serum was preincubated with 50 µg of inhibitor (cow or goat extracts) in a volume of 100 µL. After 2 hours at 37°C, 700 µL of incubation buffer was added, and strips were processed as previously described. Immunoblots and immunoblot inhibition were conducted using serum samples from patients 1 and 3.

RESULTS

Skin Tests

The results of the skin tests and specific IgE determinations are given in Table 1. Patient 1 had additional positive skin

Table 1. Results of Skin Tests and Specific IgE Determinations for Goat and Cow Dander Extracts*

	Skin Test Goat, mg/mL				Skin Test Cow, mg/mL				Specific IgE (CAP)	
	5	0.5	0.05	0.005	5	0.5	0.05	0.005	Goat	Cow
Patient 1	9	7	5	4	5	3	—	—	4.68	16.7
Patient 2	15	11	8	4	11	7	5	4	27.8	27
Patient 3	14	8	5	4	12	8	5	4	10	9.75

*Skin test results are expressed in wheal diameter (millimeters) and represent the mean diameter of the wheels; CAP values are expressed in kilounits per liter.

test reactions to dog, cat, horse, grass mix, *S kali*, *P judaica*, and *A vulgaris* and the following meats: lamb, cow, and pork. The total IgE level was 231 IU/mL. Patient 2 had additional positive skin test reactions to *O europaea*; the total IgE level was 463 IU/mL. Patient 3 had additional positive skin test reactions to horse; the total IgE level was 64 IU/mL.

Allergen Provocations

Patient 1. This patient had a positive response to challenge with the goat extract at a concentration of 5 mg/mL, showing an increase in nasal resistance of 225% (0.72 Pa s/cm³). Resistance with the buffer was 0.32 Pa s/cm³. Because this patient also reported asthma after contact with cows, a bronchial provocation test was conducted with methacholine and cow epithelium. Results of nonspecific provocation testing with methacholine were positive, with a PC₂₀ of 1.98 mg/mL. The patient had a bronchial dual reaction to cow epithelium, with an early decrease in FEV₁ (PC₂₀ of 0.48 mg/mL) and a late decrease 8 hours after challenge.

Patient 2. This patient had a positive response to challenge with the goat extract at a concentration of 0.02 mg/mL, showing an increase in nasal resistance of 354% (1.31 Pa s/cm³). Resistance with the buffer was 0.37 Pa s/cm³. This patient also underwent a bronchial provocation test with cow epithelium. He had an immediate positive reaction, with a PC₂₀ of 0.019 mg/mL of cow epithelium.

Patient 3. This patient had a positive response to challenge with the goat extract at a concentration of 5 mg/mL, showing

an increase in nasal resistance of 666% (3.33 Pa s/cm³). Resistance with the buffer was 0.5 Pa s/cm³. This patient also underwent a nasal provocation test with cow epithelium. She had a positive reaction at a concentration of 5 mg/mL, showing an increase in nasal resistance of 222% (1.11 Pa s/cm³). Resistance with the buffer was 0.5 Pa s/cm³.

Inhibition Assays and SDS-PAGE

The results of the inhibition assays are shown in Figure 1. Goat epithelium produced a strong inhibition of IgE binding to cow epithelium in patients 2 and 3 (88% and 100%, respectively), whereas in patient 1 a lower degree of inhibition was found (55%). In contrast, cow epithelium inhibited IgE binding to goat epithelium by 38% and 71% in patients 2 and 3, respectively, and by 58% in patient 1. The protein profiles of cow and goat extracts are shown in Figure 2. Several bands with a molecular weight of 9 to 70 kDa were visualized. The most prominent bands showed low molecular weights of 9 to 30 kDa in both cases.

Immunoblot and Immunoblot Inhibition

Immunoblot and immunoblot inhibition assays are shown in Figure 3. Patient 1 bound specific IgE predominantly to cow epithelium and to a lesser extent to goat epithelium. This patient recognized 4 proteins/peptides in the molecular weight range of 21 to 36 kDa in the cow extract. In the goat extract, the same patient recognized 3 bands in the molecular weight range of approximately 23 to 34 kDa. Patient 3

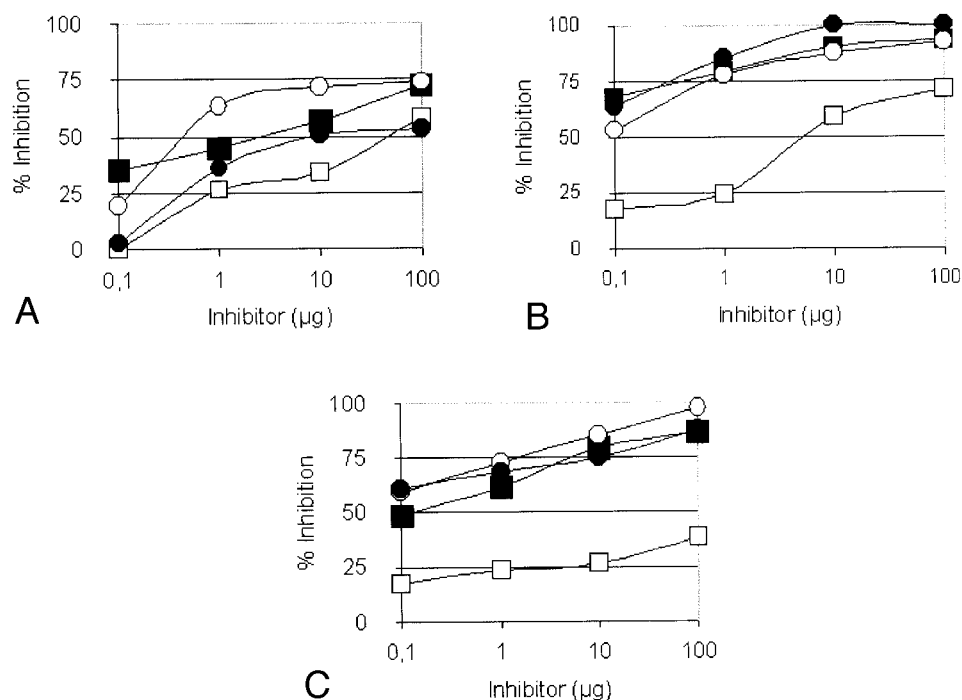


Figure 1. Enzyme allergosorbent test inhibition assay results in patients 1 (A), 2 (B), and 3 (C). Circles indicate cow epithelium disks (solid phase); inhibitors: ○ = cow epithelium and ● = goat epithelium. Squares indicate goat epithelium disks (solid phase); inhibitors: □ = cow epithelium and ■ = goat epithelium.

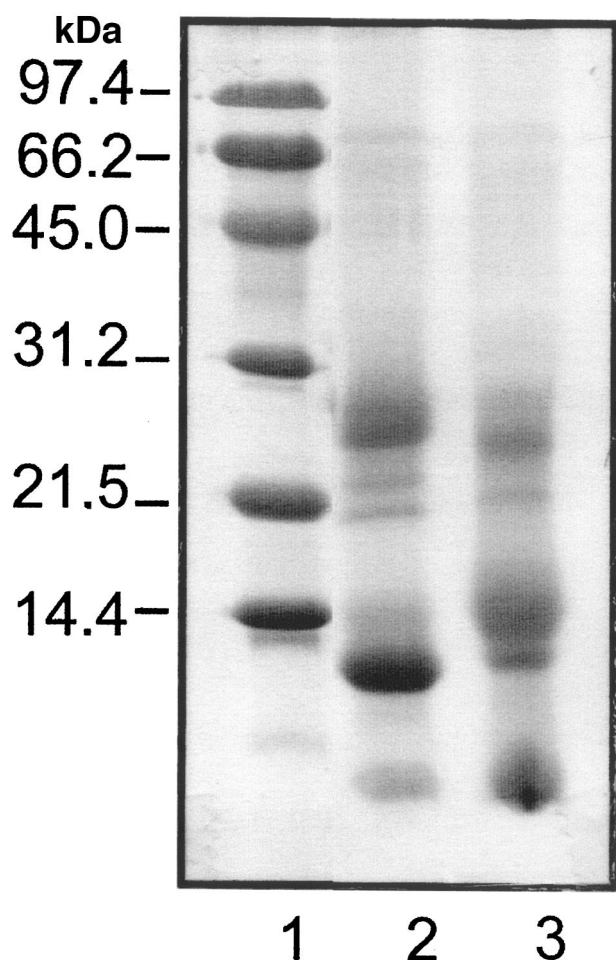


Figure 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of cow and goat extracts. Lane 1, molecular marker; lane 2, cow epithelium; and lane 3, goat epithelium.

recognized several bands in both extracts. Four bands were detected in the cow extract in the molecular weight range of 26 to 53 kDa, and 4 bands were detected in the goat extract in the molecular weight range of 24 to 36 kDa.

The immunoblot inhibition assays also showed variable degrees of inhibition. Preincubation with goat extract produced a partial inhibition of specific IgE binding to cow epithelium using serum samples from patient 1. One band of approximately 21 kDa was not inhibited. In patient 3, the goat epithelium extract completely inhibited specific IgE binding to cow epithelium, whereas the cow extract only partially inhibited IgE binding to the goat extracts, persisting a 24-kDa band.

DISCUSSION

We described 3 patients who are occupationally exposed to goat and cow epithelia and have clinical symptoms of rhinitis and asthma on exposure. All the patients have frequent con-

tact with these animals and have developed a marked sensitivity to their allergens, as demonstrated by skin testing, specific IgE determinations, organ-specific provocations, and immunoblots. All 3 patients had a positive provocation test result, either nasal or bronchial, with the offending allergen. These results demonstrate good correlation among the clinical history, exposure, and the laboratory findings for goat and cow allergens. It has been demonstrated that cow allergens can be detected in large quantities in the air in barns and other occupational settings, and, therefore, cow epithelium can be considered an important aeroallergen.²⁴ Less information is available about the aerodynamic properties of goat allergens, although patient 2 reported respiratory symptoms while in the proximity of the goat farm and fresh goat's milk, suggesting the presence of airborne goat allergens.

Patient 1 had higher specific IgE levels to cow than to goat. This patient was challenged with both allergens, obtaining a positive reaction in both cases. Because the patient reported asthma after contact with cows, he underwent a bronchial challenge, resulting in a strong positive reaction. This patient also mentioned being more in contact with cows than with goats. Results of enzyme allergosorbent test (EAST) inhibition assays suggest that this patient is mainly sensitized to cow allergens because the goat extract produces only marginal inhibition of the cow extract.

Patient 2 had detectable specific IgE levels to cow and goat extracts and a strong positive challenge with both extracts, and small doses of both allergens produced a positive result in both challenge tests. The patient reported asthma symptoms after exposure to cows, and results of a provocation challenge with cow extract were also positive. The EAST inhibition assays using his serum samples showed that the goat extract completely inhibited IgE binding to the cow extract. However, the cow extract partially inhibited IgE binding to the goat extract. In this case, sensitivity to both allergens seems to be clinically relevant.

Patient 3 also had detectable specific IgE levels to goat and cow. She reported intermittent exposure to both animals. Nasal provocation test results were positive with both extracts, although less allergen was required in the nasal provocation with goat epithelia than with cow epithelia. Results of the EAST inhibition assays demonstrated that the goat extract completely inhibited IgE binding to the cow extract and that the cow extract did not inhibit the goat extract. The immunoblot experiments demonstrated similar results, suggesting that the patient was mainly sensitized to goat allergens. Because cows, goats, and sheep belong to the Bovidae family, some cross-reactivity should be expected, as has been demonstrated in our experiments. However, although cow allergens have been studied in detail, no studies have been conducted with goat allergens. The presence of panallergens in animal epithelial extracts, such as lipocalins, is common, and, therefore, they should also be present in goat extracts.

Cross-reactivity among the milk of cows, goats, and sheep has also been suggested.^{25,26} Owing to the increasing rate of sensitization to animal danders in occupationally and nonoc-

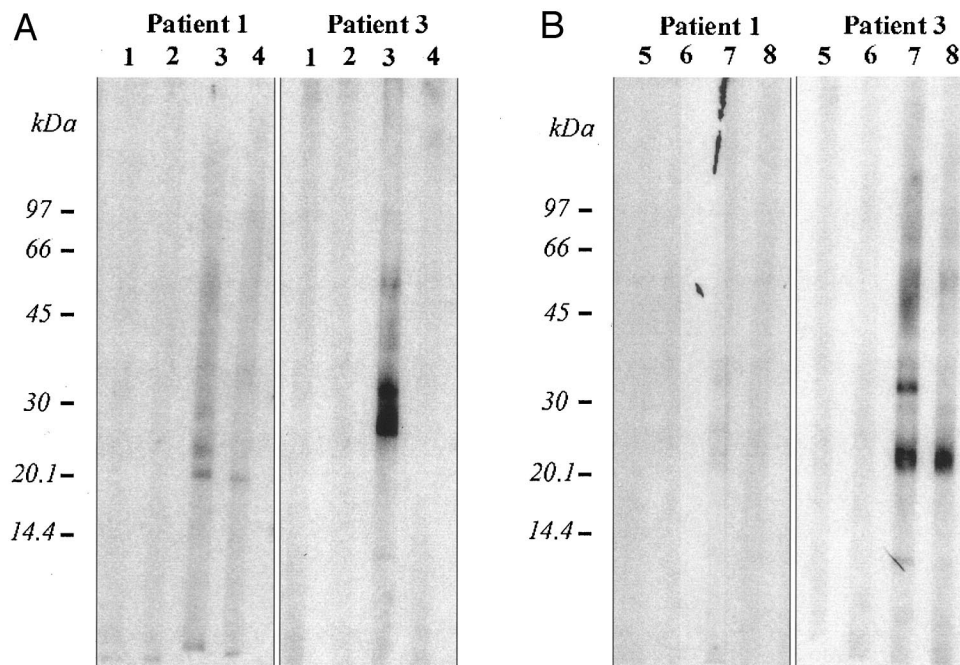


Figure 3. Immunoblot and immunoblot inhibition experiments. A, Cow epithelium. Lane 1, blank; lane 2, cow epithelium; lane 3, phosphate-buffered saline; and lane 4, goat epithelium. B, Goat epithelium. Lane 5, blank; lane 6, goat epithelium; lane 7, phosphate-buffered saline; and lane 8, cow epithelium.

cupationally exposed individuals, standardized extracts are needed to better diagnose and treat these patients. Based on the clinical and laboratory findings, these patients, who cannot avoid contact with the allergen, could be good candidates for allergen immunotherapy.

In conclusion, we confirmed clinical sensitivity and specific IgE to cow and goat allergens in 3 occupationally exposed individuals. To our knowledge, these are the first cases of occupational sensitization to goat allergens described in the recent indexed literature. We also identified moderate-to-high cross-reactivity between goat and cow allergens. Further studies should be conducted to identify the nature of the allergens that are responsible for the cross-reactivity between these mammalian species.

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