

Results of intradermal tests in horses without atopy and horses with atopic dermatitis or recurrent urticaria

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Objective—To compare results of intradermal tests (IDT) for environmental allergens at 30 minutes and 4, 6, and 24 hours after injection in horses without atopy and horses with atopic dermatitis (AD) or recurrent urticaria (RU).

Animals—22 horses without atopy, 10 horses with RU, and 7 horses with AD.

Procedure—In all horses, medical history was obtained, and results of physical examination, hematologic examination, serum biochemical analyses, examination of bronchoalveolar lavage fluid, and IDT with 73 allergens were examined.

Results—Horses with AD or RU had a significantly greater mean number of positive reactions for IDT, compared with horses without atopy. Horses with AD had a significantly greater number of positive reactions than horses without atopy in every allergen group at all time periods, except for molds at 4 and 24 hours. Horses with RU had a significantly greater number of positive reactions than horses without atopy in every allergen group, except for molds at 30 minutes and 4 and 6 hours, trees at 4 and 6 hours, and grasses at 4 hours.

Conclusions and Clinical Relevance—A significantly greater number of positive reactions for IDT in horses with AD or RU, compared with horses without atopy, provides evidence of type-I IgE-mediated hypersensitivity for these diseases. Evaluation of results of IDT performed in horses with AD or RU is useful in determining specific allergens for the formulation of immunotherapy along with providing identification of allergens that could be useful when creating avoidance strategies. (*Am J Vet Res* 2001;62:1051–1059)

Atopy is defined as a familial hypersensitivity of skin and mucous membranes to environmental allergens associated with increased IgE production or altered nonspecific reactivity in various organ systems (eg, skin in the case of atopic dermatitis [AD] and lungs in the case of asthma).¹ Classically, atopy is an immediate or type-I hypersensitivity¹ with increased

IgE production, T-cell dysregulation, increased releasability of mediator-secreting cells, and organ hyperreactivity. The immediate reaction develops after exposure to an allergen. Mast cells bearing IgE directed against the relevant allergen release various mediators, cytokines, and leukocyte chemotactic factors into local tissue within 15 to 60 minutes after exposure to that allergen.¹ In humans, 4 to 6 hours after the immediate reaction begins to subside, there is the onset of an IgE-dependent late-phase reaction (LPR) characterized initially by expression of leukocyte adhesion molecules on postcapillary venous endothelium, which is followed by infiltration of eosinophils, neutrophils, and mononuclear cells.² By 24 hours after onset of the LPR, the cellular infiltrate consists predominantly of mononuclear cells.^{1,3} The clinical importance or correlation of severity of disease and the LPR or delayed reaction has not been determined in domestic animals.

In humans, atopy is associated with varied expression of at least 20 genes in addition to the influence of multiple environmental factors. Thus, it is not surprising that as a result of genotypic and phenotypic variability, each atopic person possesses a unique allergic fingerprint, resulting in varied clinical manifestations.⁴

Atopy in horses is primarily cutaneous or respiratory in origin and may be a combination of both.⁵⁻¹⁰ Atopic dermatitis and recurrent urticaria (RU) are 2 cutaneous diseases associated with IgE-mediated hypersensitivity.^{4,11} Pruritus is a quintessential feature of AD and is the primary clinical sign of AD in all species.^{1,5,6} Clinical signs specific for horses with AD are not clearly defined, and multiple manifestations have been attributed to AD on the basis of results of intradermal tests (IDT) and response to immunotherapy.⁵ Most gross cutaneous changes are secondary to pruritic-induced scratching and may include alopecia, excoriations, lichenification, and hyperpigmentation of the face, ears, neck, mane, ventrum, tail, and legs.^{5,6,12}

The pathogenesis of RU is not completely understood, but IgE-mediated mast cell degranulation is believed to be of central importance in coordinating the urticarial response.¹¹ Clinical manifestations vary from those of a minor transitory nature to major systemic and possibly life-threatening anaphylaxis. Onset can be peracute or acute following exposure to the instigating factor. Lesions usually resolve within 12 to 24 hours; however, an affected horse may have persistent and chronic eruption of lesions over a period of days to months. Thus, lesions may exist at various stages of formation and resolution.¹² Urticaria is char-

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acterized by the appearance of edematous lesions of the skin or mucous membranes, which are termed wheals. Wheals often are sharply circumscribed raised papules or nodular lesions that appear white or pink in nonpigmented skin, are flat-topped with steep-walled sides, and typically pit when pressure is applied. Lesions vary in size and shape.¹² Alopecia usually is not evident unless some of the lesions are exudative. Recurrent urticaria can cause pruritus but not always.⁶ Both AD and RU have been reported worldwide, but epidemiologic studies to indicate the incidence or prevalence of these cutaneous allergic diseases in horses are lacking. Anecdotal reports^{5,6,10} suggest that cutaneous allergies are a widespread problem.

Intradermal testing is an accepted method of detecting IgE-mediated allergen hypersensitivity in atopic humans,¹³ dogs, cats,¹⁴ and horses.¹⁵ Intradermal testing has been regarded as the criterion-referenced standard for identification of allergens to be included in immunotherapy vaccines in horses.¹⁰ During IDT, horses with AD and RU have immediate, late-phase, and delayed reactions to allergens commonly found in the environment.¹⁵⁻¹⁷ However, horses without atopy may have positive results on IDT when tested with those same allergens.^{16,18} Thus, it may be difficult to interpret the clinical relevance of positive reactions on IDT.

Several studies¹⁵⁻²² have revealed IDT reactions to individual and group extracts of insects, molds, tree pollens, weed pollens, and grass pollens in clinically nonaffected horses and horses with documented AD, RU, or hypersensitivity to insects. Most of those studies have focused on determining the appropriate threshold concentrations of commercially available allergens and on reactivity to insect allergens in clinically normal horses and horses hypersensitive to insects.^{16,18,20-22} To our knowledge, there have not been any published reports that document IDT reactivity patterns or odds ratios for reactivity to specific allergens among horses without atopy and horses with 2 distinct clinical manifestations of cutaneous allergic disease, namely AD and RU. In addition, the incidence and relationship among immediate reactions, LPR, and delayed reactions for specific allergen groups during IDT in horses without atopy and horses with AD or RU have not been reported.

The objective of the study reported here was to use IDT to compare reactivity to specific environmental allergens 30 minutes and 4, 6, and 24 hours after exposure in horses without atopy and horses with AD or RU. Our goal was to identify differences in patterns of reactivity among the 3 groups of horses that may be of diagnostic relevance in the determination of the role of type-I allergic hypersensitivity in horses with AD or RU.

Materials and Methods

Sample population—Horses without atopy were selected for the study from horses donated to the College of Veterinary Medicine, The Ohio State University. Horses with AD or RU horses were selected from horses referred to the Veterinary Teaching Hospital, The Ohio State University for evaluation of dermatologic disease. The study was performed from July 1998 to August 1999. The IDT were performed throughout this period in all 3 groups of horses and were not

clustered or limited to any particular month. A detailed history was obtained for at least the preceding 12 months for each horse, and it included specific questions regarding previous or current dermatologic and respiratory tract disease. Because atopy may be a seasonal or nonseasonal disease, responses to the questions allowed identification and exclusion of horses that may have had seasonal atopy but that did not have clinical signs at the time of examination. All horses were given routine treatment with anthelmintics prior to the study.

When a horse with AD or RU had reactivity to insect allergens alone during the IDT, it was excluded from the study. However, when a horse with AD or RU had reactivity to aeroallergens as well as insect allergens during IDT, it was included in the study. All horses with AD or RU had clinical signs of active disease at the time that they entered the study.

All procedures were performed in accordance with guidelines established by an institutional laboratory animal care and use committee. The study was approved by the College of Veterinary Medicine, The Ohio State University.

Horses without atopy—Twenty-two horses (14 geldings, 7 mares, and 1 stallion) were selected for inclusion in the study on the basis of a lack of historical and clinical evidence suggestive of allergic disease of any organ system. Atopic disease is defined as a familial hypersensitivity of skin and mucous membranes to environmental allergens associated with increased IgE production or altered nonspecific reactivity in various organ systems (eg, skin in the case of atopic dermatitis and lungs in the case of asthma).¹ On the basis of that definition, this group was classified as horses without atopy. These horses were not considered completely healthy, because they had been donated to our facility as a result of osteoarthritis (4 horses), laminitis (3), chronic endometritis (2), abdominal cryptorchidism (1), navicular disease (4), and developmental contracture of the superficial digital flexor tendons, sesamoiditis, cervical vertebral instability, occipito-atlanto-axial malformation, arthrogryposis, idiopathic peripheral neuropathy characterized by tremors of the musculature of the pelvic limbs and elevation of the tail (shivers), exertional rhabdomyolysis, and behavior problems (1 horse each).

Breeds represented included Arabian (1 horse), Bashkir Curly (1), Oldenburg (1), Percheron (1), Quarter Horse (6), Standardbred (2), and Thoroughbred (10). Additional criteria for horses without atopy included a respiratory rate of < 24 breaths/min, lack of crackles or wheezes heard during rebreathing examination, and lack of cough on physical examination. Prior to inclusion in the study, horses without atopy were housed separately in stalls for 36 hours to 1 week to serve as a natural challenge (eg, hay and straw)²³ to eliminate the possibility of undetected respiratory tract disease.

Horses with AD—Seven horses (3 geldings, 3 mares, and 1 stallion) with historical and clinical signs compatible with seasonal and nonseasonal AD^{5,6} and with active clinical disease were selected for the study. Breeds represented included American Saddlebred (2 horses), Arabian (2), Belgian (1), Quarter Horse (1), and Tennessee Walking Horse (1).

Horses with RU—Ten horses (5 geldings, 4 mares, and 1 stallion) with historical and clinical signs compatible with pruritic seasonal and nonseasonal RU and with active clinical disease were selected for the study. Breeds represented included Dutch Warmblood (1 horse), grade (1), Paint (1), and Quarter Horse (7).

Animal housing—All horses were housed separately in 4 × 4-m stalls. Stalls were designed to allow free airflow and recirculation of air via an air conditioner or ventilation with

outside air. All horses were fed grain and mixed-grass hay, and they were bedded on straw. Stalls were cleaned once each day. All horses were allowed ad libitum access to water.

Diagnostic procedures—All horses were subjected to a complete physical examination including auscultation of the chest during quiet breathing and forced rebreathing. Diagnostic tests performed on all horses included hematologic examination with differential cell count, serum biochemical analysis, cytologic examination of **bronchoalveolar lavage (BAL)** fluid, and an IDT. Specimens of skin surface were submitted for cytologic examination, bacterial culture, and fungal culture, and skin biopsy specimens were submitted for histologic examination or evaluation of an elimination-diet food trial prior to entry into the study for horses suspected of having infectious skin disease or food hypersensitivity. These additional diagnostic procedures were used to rule out differential diagnoses for pruritic or nonpruritic dermatitis that may have masqueraded as AD or allergic RU.

Hematologic testing—Blood samples were collected by jugular venipuncture into sterile glass tubes containing EDTA for CBC and sterile glass serum tubes for serum biochemical analyses. The CBC was performed, using an automated cell counter,^a and a differential count of 100 consecutive WBC was performed manually. Serum biochemical analyses were performed, using an automated serum analyzer.^b

Respiratory tract examination—Physical examination of the respiratory system was performed on all horses by 1 investigator (GL) before BAL was performed. Physical examination was conducted while horses were at rest and consisted of auscultation of the thorax during quiet breathing and forced breathing induced by the use of a rebreathing bag. Inclusion criteria for all horses were a respiratory rate of < 24 breaths/min, lack of crackles or wheezes during rebreathing examination, and lack of cough during physical examination.

Collection of BAL fluid—Horses were sedated with xylazine hydrochloride^c (0.5 mg/kg of body weight, IV). An 8-mm-diameter, 2.5-m-long silicone equine BAL tube^d was inserted into the nasal cavity, advanced into the trachea to the level of the carina and wedged in a subsegmental bronchus of a diaphragmatic lung lobe. Lavage consisted of infusion and immediate aspiration of a 150-ml aliquot of lukewarm sterile saline (0.9% NaCl) solution. Manual aspiration of the BAL fluid was achieved through use of a series of sterile 60-ml syringes. The BAL fluid was transported to our laboratory in a sterile glass tube containing EDTA and was processed immediately after collection. Total concentration of nucleated cells in BAL fluid was measured, using an automated cell counter.^a For each BAL sample, 1 slide was prepared, using cytocentrifugation.^e That slide was stained with Wright-Giemsa stain, and 100 consecutive nucleated cells were counted. The cytologist was not aware of the origin of the slides (ie, horses without atopy or horses with AD or RU). Calculation of cell concentrations in BAL fluid was obtained by multiplying the percentage of each cell type by the total number of nucleated cells observed.

IDT procedure—Prior to IDT, a minimum of 4 weeks was allowed to elapse from the last administration of non-steroidal anti-inflammatory drugs, glucocorticoids, antihistamines, bronchodilators (sympathomimetics or methyl xanthine derivatives), acepromazine, or other phenothiazine tranquilizers. Intradermal testing was performed immediately after the BAL procedure while the horses were still sedated. Hair was clipped from a rectangular area (30 × 15 cm) on the lateral aspect of the neck above the jugular furrow halfway between the mandible and shoulder and 1 hand width below the base of the mane. Skin was disinfected with

a solution of 70% alcohol to eliminate gross contamination. A permanent marking pen was used to indicate injection sites. Injections were made above and below each mark and were spaced at a distance of 5 cm. A positive-control injection (histamine,^f 0.05 ml) at a concentration of 1:100,000 (wt:vol) and a negative-control injection (phosphate-buffered allergen diluent,^g 0.05 ml) were administered to each horse.

Concentrated test allergens were purchased from a commercial allergy laboratory,^h and dilutions were prepared with phosphate-buffered allergen diluent^g in accordance with standard protocols.^{13,14} Seventy-three specific allergenic extracts consisting of individual species and species-related mixes were tested, including 24 molds, 11 weeds, 16 trees, 15 grasses, and 7 insects. Allergen selection was based on known and perceived importance of allergens in the midwestern United States. Extract of *Culicoides* spp was not available at the beginning of the study; therefore, it was not used for testing in 7 horses without atopy, 2 horses with AD, and 5 horses with RU. Grain mill dust mix was classified as a mold, because airborne spores and other fungal particles are universally detected over landscapes, especially field crops, and often form the bulk of suspended biogenic debris typical of a dust.²⁴ Optimal IDT allergen dilutions have not been evaluated and standardized for horses; therefore, we selected allergen dilutions on the basis of results for IDT studies reported for horses.^{16,18,20,22} All allergens were tested at a dilution of 1,000 protein nitrogen units (PNU)/ml, except insect extracts, grain mill dust mix, and grain smut mix extracts, which were tested at a dilution of 500 PNU/ml. However, black ant extract was tested at a dilution of 1,000 PNU/ml. An aliquot (0.05 ml) of each allergen was injected intradermally, using a 26-gauge needle. Injection sites were evaluated 30 minutes after administration for immediate reactions, 4 and 6 hours after administration for late-phase reactions, and 24 hours after administration for delayed reactions. Reactions were judged subjectively on a scale of 0 to 4 (0 was equal to the reaction for the diluent injection, and 4+ was equal to the reaction for the histamine injection).¹³ Intermediate reactions were graded as 1+, 2+, or 3+. Criteria for the subjective score included erythema, induration, and diameter of the wheal. Reactions of ≥ 2+ were considered positive.¹⁴ All reactions in all horses were subjectively classified by 1 investigator (GL).

Necropsy and histologic examination—After IDT scores were recorded at 24 hours, all horses without atopy were sedated by administration of xylazine^c (0.5 mg/kg, IV). A 14-gauge catheterⁱ was inserted into a jugular vein, and euthanasia solution^j was injected (1.0 ml/4.5 kg, IV). During necropsy, the lungs were physically examined, and tissue specimens were collected from the left dorsal apical region. Specimens were fixed in phosphate-buffered 10% formalin, embedded in paraffin, sectioned at a thickness of 3 to 5 μ m, and stained with H&E, periodic acid Schiff, Alcian blue, Masson trichrome, and neutral red stains. A single pathologist categorized each specimen as normal or abnormal on the basis of a classification scheme that was devised by ranking the known histologic features of **chronic obstructive pulmonary disease (COPD)**, using a 2-point scale.²⁵

Statistical analysis—Data were analyzed, using statistical analytic software.^{26,k} Odds ratios were estimated by use of χ^2 analysis,²⁷ using another statistical software package.^l Disease odds ratio is the odds of atopic disease in horses with AD or RU, given an exposure to an allergen group, compared with the odds of atopic disease in the horses without atopy.²⁸ Analysis of data for BAL fluid was conducted by use of a fixed-effects multiple regression model to determine whether there were significant differences in the percentage of neu-

Table 1—No. of positive reactions to weed allergens at each time point during intradermal tests (IDT) in 22 horses without atopy, 7 horses with atopic dermatitis (AD), and 10 horses with recurrent urticaria (RU)

Allergen	Time after injection of allergens											
	0			30 min			4 h			6 h		
	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU
Cocklebur	8	4	4	11	5	4	7	4	2	0	0	1
Dock-sorrel mix	9	4	6	14	4	8	8	3	5	0	0	1
Firebush or Kochia	8	5	7	6	4	5	2	3	4	0	0	1
Goldenrod	7	4	5	1	3	3	0	2	2	0	0	2
Hemp, western water	5	2	5	10	6	7	3	3	7	0	0	0
Lamb's-quarter	6	4	4	2	2	3	0	2	3	0	0	1
Marsh elder, rough	7	4	6	2	2	4	0	1	3	0	0	1
Pigweed, rough	5	2	6	4	2	1	0	0	2	0	0	0
Plantain, English	7	1	6	2	0	2	0	0	2	0	0	1
Ragweed mix	7	4	6	6	3	4	2	3	2	0	0	0
Russian thistle	9	4	7	4	4	4	2	3	3	0	0	0

Table 2—No. of positive reactions to grass allergens at each time point during IDT in 22 horses without atopy, 7 horses with AD, and 10 horses with RU

Allergen	Time after injection of allergens											
	0			30 min			4 h			6 h		
	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU
Alfalfa	7	1	5	9	1	2	4	0	2	0	0	0
Bermuda	7	2	5	2	1	3	1	1	2	0	1	1
Fescue, meadow	4	3	5	2	3	1	2	2	1	0	1	0
Kentucky blue/June Johnson	5	5	5	2	4	1	1	2	0	0	1	0
	4	0	5	1		3	0	0	2	0	0	0
Oat, cultivated	1	4	4	2	2	1	0	2	1	0	0	0
Orchard	3	2	2	0	1	1	1	1	1	0	1	0
Red top	4	4	4	2	2	0	1	2	1	0	0	1
Rye, cultivated	2	3	5	3	1	1	3	0	1	0	0	0
Rye, perennial	6	4	6	5	1	1	4	1	1	0	0	0
Sage, pasture	6	3	2	1	1	1	1	1	1	0	0	0
Smooth brome	3	2	2	0	1	2	1	1	2	0	0	1
Sweet vernal	7	2	3	2	1	2	1	1	2	0	1	0
Timothy	9	3	5	4	1	2	1	1	1	0	1	0
Wheat, cultivated	6	3	4	2	1	2	0	1	2	0	0	0

Table 3—No. of positive reactions to tree allergens at each time point during IDT in 22 horses without atopy, 7 horses with AD, and 10 horses with RU

Allergen	Time after injection of allergens											
	0			30 min			4 h			6 h		
	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU
Ash mix	5	2	4	2	1	1	0	1	1	0	0	1
Beech	6	2	3	3	1	1	3	0	1	0	0	1
Birch mix	5	2	3	4	2	2	2	1	2	0	0	1
Box elder	4	3	2	1	1	2	0	1	2	0	1	1
Cedar, red	4	3	4	0	1	1	0	1	1	0	0	1
Cottonwood	5	2	2	1	0	2	0	0	1	0	0	0
Elm mix	3	4	3	1	3	1	0	1	0	0	0	0
Hazelnut	3	2	1	2	0	0	2	0	0	0	0	0
Hickory mix	2	2	5	0	1	2	0	1	1	0	0	1
3 Maple mix	6	6	6	1	2	3	1	0	1	0	0	1
Eastern oak mix	2	4	4	0	1	1	0	1	0	0	0	0
Mulberry, white	5	2	3	2	2	1	1	2	1	0	0	0
Pine, white	4	2	3	0	1	0	0	2	0	0	0	0
Sycamore, east	4	2	5	2	1	1	1	1	0	0	0	1
Walnut, black	2	2	3	1	1	0	1	2	0	0	0	0
Willow, black	8	2	2	11	6	3	5	4	2	0	0	1

Table 4—No. of positive reactions to mold allergens at each time point during IDT in 22 horses without atopy, 7 horses with AD, and 10 horses with RU

Allergen	Time after injection of allergens											
	30 min			4 h			6 h			24 h		
	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU
<i>Alternaria tenuis</i>	1	1	0	1	1	0	0	0	0	0	0	0
<i>Aspergillus mix</i>	1	1	2	0	0	0	0	0	0	0	0	0
<i>Botrytis cinerea</i>	1	1	1	1	0	0	1	0	0	0	0	0
<i>Candida albicans</i>	3	1	0	6	2	1	7	2	1	10	2	4
<i>Cephalosporium acremonium</i>	3	1	1	1	1	1	0	1	1	0	0	1
<i>Cladosporium herbarum</i>	2	0	1	1	0	0	0	0	0	0	0	0
<i>Curvularia spicifera</i>	1	0	0	1	0	0	0	0	0	0	0	0
<i>Epicoccum purpurascens</i>	0	1	1	0	0	0	0	1	0	0	0	0
<i>Fusarium mix</i>	2	1	0	1	1	0	0	1	1	0	0	0
<i>Geotrichum candidum</i>	3	2	3	8	4	3	3	3	2	0	0	2
Grain smut mix	9	4	5	18	5	10	13	5	8	0	1	1
Grain mill dust mix	13	6	8	12	5	4	11	5	4	0	0	2
<i>Helminthosporium sativum</i>	2	1	2	1	0	0	0	0	0	0	0	0
<i>Hormodendrum hordei</i>	0	1	0	1	0	0	1	0	0	0	1	0
<i>Monilia sitophila</i>	3	3	1	0	1	0	0	0	0	0	0	2
<i>Mucor mix</i>	3	2	5	1	0	1	0	0	0	0	0	0
<i>Penicillium mix</i>	2	1	1	0	1	0	0	1	0	0	0	1
<i>Phoma betae</i>	1	1	1	4	0	0	2	0	0	0	0	0
<i>Pullularia pullulans</i>	1	1	2	0	0	2	0	0	0	1	0	0
<i>Rhizopus mix</i>	7	2	4	3	1	4	4	3	4	3	0	2
<i>Rhodotorula rubra</i>	1	1	0	1	1	1	0	2	0	0	0	1
<i>Saccharomyces cerevisiae</i>	2	2	0	3	2	0	4	2	0	0	0	0
<i>Stemphylium solani</i>	1	1	1	1	1	0	1	1	0	0	0	0
<i>Trichoderma viride</i>	1	0	1	1	0	0	0	1	0	0	0	0

Table 5—No. of positive reactions to insect allergens at each time point during IDT in 22 horses without atopy, 7 horses with AD, and 10 horses with RU

Allergen	Time after injection of allergens											
	30 min			4 h			6 h			24 h		
	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU	Without atopy	AD	RU
Black ant	12	6	9	12	6	7	9	5	7	0	0	1
Black fly	11	3	5	4	3	2	1	1	4	0	0	1
Culicoides spp	1	1	2	1	1	1	0	1	1	0	0	0
Deer fly	9	3	6	3	1	2	1	1	3	0	0	3
Flea	4	2	4	1	1	3	0	0	2	0	0	0
Horse fly	10	6	6	7	3	2	4	4	1	0	0	0
Mosquito	11	4	7	5	5	3	2	4	4	0	0	0

trophils, total number of WBC, and age among horses without atopy or horses with AD or RU.^{26,k} Differences were considered significant at a value of $P \leq 0.05$ for all tests.

Results

Age, CBC, serum biochemical analyses, and respiratory tract examination—We did not detect significant differences in age among horses without atopy (mean $\pm$ SD, 5.9 ± 4.1 years) and horses with AD (11 ± 5.7 years) or RU (7.9 ± 5.0 years). Values for CBC and serum biochemical analyses of all horses were within reference ranges for our laboratory. Results of respiratory tract examinations were normal in all horses, and evidence of skin disease was not detected in horses without atopy.

Cytologic examination of BAL—The proportion of neutrophils observed in BAL fluid was $\leq 17\%$ of the recovered cells in all horses, which was within the limits of neutrophils reported for control horses (0 to

17%).²⁹⁻³¹ We did not detect significant differences in the differential percentages of neutrophils among horses without atopy ($9.1 \pm 8.0\%$) and horses with AD ($6.0 \pm 4.5\%$) or RU ($8.0 \pm 6.1\%$).

Results of IDT—The greatest number of positive reactions in horses without atopy was observed for grain smut mix ($n = 40$), grain mill dust mix (36), black ant extract (33), dock-sorrel (31), cocklebur (26), and *Candida albicans* (26). In these horses, there were 88 possible reactions (22 horses $\times$ 4 observation times) for each allergen tested (Tables 1–5). The greatest number of positive reactions in horses with AD was observed for black ant extract ($n = 17$), grain mill dust mix (16), grain smut mix (15), horse fly (13), and cocklebur (13). In these horses, there were 28 possible reactions (7 horses $\times$ 4 observation times) for each allergen tested. The greatest number of positive reactions in horses with RU was observed for grain smut mix ($n = 24$), black ant extract (24), dock-sorrel (19),

western water hemp (19), and grain mill dust mix (18). In these horses, there were 40 possible reactions (10 horses $\times$ 4 observation times) for each allergen tested.

All horses had positive reactions to 3 or more allergens. Positive reactions were recorded most commonly at 30 minutes in horses without atopy (341/709, 48%), horses with AD (181/421, 43%), and horses with RU (255/540, 47%; Table 6). Mean number of reactions in horses with AD or RU was significantly greater than for horses without atopy at each of the time points as well as for the cumulative number of positive reactions of all time points. Horses without atopy had a mean of 32 positive reactions for all time points (95% confidence interval (CI), 17.2 to 47.3). Four of 7 horses with AD and 6 of 10 horses with RU had $\geq$ 48 positive reactions for all time points. At 30 minutes, horses without atopy had a mean of 16 positive reactions (95% CI, 13.4 to 17.6 positive reactions). Five of 7 horses with AD and 6 of 10 horses with RU had $\geq$ 18 positive reactions at 30 minutes.

A positive reaction at 4 hours was preceded by a positive reaction at 30 minutes for 141 of 229 (62%) reactions in horses without atopy, 94 of 127 (74%)

reactions in horses with AD, and 112 of 136 (82%) reactions in horses with RU. A positive reaction at 6 hours was preceded by a positive reaction at 30 minutes or 4 hours in 119 of 125 (95%) reactions in horses without atopy, 91 of 102 (89%) reactions in horses with AD, and 105 of 111 (95%) reactions in horses with RU. Positive reactions at 24 hours in horses without atopy accounted for 14 of 709 (2%) of all positive reactions and were exclusive to mold allergens (*C albicans*, *Rhizopus* mix, and *Pullularia pullulans* (Table 4). Positive reactions at 24 hours also were uncommon in horses with AD, accounting for 11 of 421 (3%) of all positive reactions; they were recorded most commonly for grass allergens (Table 2). Of the 3 groups of horses, horses with RU had the greatest mean number and percentage of positive reactions at 24 hours, accounting for 38 of 540 (7%) positive reactions. These reactions were spread fairly evenly among all allergens, except for grass allergens, which rarely caused a positive reaction at 24 hours. Positive reactions at 24 hours that were not preceded by a positive reaction at an earlier time point accounted for 2 of 421 (0.5%) reactions in horses with AD and 10 of 540 (2%) reactions in horses with RU. Molds accounted for 10 of these reactions, and cocklebur and Bermuda grass accounted for the remaining 2 reactions.

Odds ratios for the likelihood of having a positive reaction to each allergen group at each time point were calculated (Table 7). Horses with AD were likely to have a significantly greater number of positive reactions than horses without atopy for every allergen group at every time point, except for mold allergens at 4 and 24 hours. Horses with RU were likely to have a significantly greater number of positive reactions than horses without atopy for every allergen group at every time point, except for mold allergens at 30 minutes and 4 and 6 hours, tree allergens at 4 and 6 hours, and grass allergens at 4 hours.

Necropsy and histologic examination of lungs— Findings for the thorax during gross examination and histopathologic scores of $\leq$ 0.2 (out of a possible score of 2) for lung parenchyma in all categories confirmed a

Table 6—No. of positive reactions during IDT of 22 horses without atopy, 7 horses with AD, and 10 horses with RU

Time after injection of allergens*	Horses without atopy	Horses with AD	Horses with RU
0 min†	341 (16 $\pm$ 5.6)	181 (26 $\pm$ 6.0)‡	255 (26 $\pm$ 8.0)‡
4 h†	229 (10 $\pm$ 0.4)	127 (18 $\pm$ 2.0)‡	136 (14 $\pm$ 7.0)‡
6 h†	125 (6 $\pm$ 1.2)	102 (15 $\pm$ 3.0)‡	111 (11 $\pm$ 6.6)‡
24 h†	14 (1 $\pm$ 0.1)	11 (2 $\pm$ 1.0)‡	38 (4 $\pm$ 2.2)‡
All time points†	709 (32 $\pm$ 22)	421 (60 $\pm$ 19)‡	540 (54 $\pm$ 4)‡

Numbers in parentheses represent mean $\pm$ SEM No. of positive reactions per horse.
 **Culicoides* extract was not tested in 7 horses without atopy, 2 horses with AD, and 5 horses with RU. †No. of possible reactions for each time point for horses without atopy, horses with AD, and horses with RU was 1,599, 509, and 725, respectively, whereas No. of possible reactions for all time points for horses without atopy, horses with AD, and horses with RU was 6,396, 2,036, and 2,900, respectively. ‡Value is significantly ($P \leq 0.05$) different from value for horses without atopy.

Table 7—Percentage of positive reactions* during IDT in horses without atopy, horses with AD, and horses with RU

Allergen	Group	Time after injection of allergens															
		30 min				4 h				6 h				24 h			
		%*	P	OR	CI	%	P	OR	CI	%	P	OR	CI	%	P	OR	CI
Grass	Without Atopy	22.4	—	1	—	11.2	—	1	—	6.4	—	1	—	0.0	—	1	—
	AD	39.0	< 0.001	2.22	1.35–3.64	20.0	0.021	1.98	1.05–3.70	15.2	0.004	2.65	1.25–5.56	5.7	ND	ND	ND
	RU	41.3	< 0.001	2.44	1.58–3.77	15.3	0.205	1.43	0.79–2.60	13.3	0.011	2.26	1.13–4.52	2.0	ND	ND	ND
Weed	Without Atopy	32.2	—	1	—	25.6	—	1	—	9.9	—	1	—	0.0	—	1	—
	AD	49.4	0.006	2.05	1.18–3.57	45.5	0.001	2.42	1.37–4.27	31.2	< 0.001	4.11	2.07–8.19	0.0	ND	ND	ND
	RU	56.4	< 0.001	2.72	1.67–4.44	40.9	0.004	2.01	1.21–3.33	31.8	< 0.001	4.24	2.28–7.91	7.3	ND	ND	ND
Tree	Without atopy	19.3	—	1	—	8.8	—	1	—	4.6	—	1	—	0.0	—	1	—
	AD	37.5	< 0.001	2.51	1.53–4.10	21.4	0.003	2.82	1.52–5.26	16.1	< 0.001	4.02	1.87–8.67	.89	ND	ND	ND
	RU	33.1	0.001	2.07	1.33–3.23	13.1	0.134	1.56	0.83–2.93	8.1	0.104	1.86	0.82–4.20	5.0	ND	ND	ND
Mold	Without atopy	11.9	—	1	—	12.5	—	1	—	8.9	—	1	—	2.7	—	1	—
	AD	20.8	0.004	1.94	1.20–3.14	15.5	0.321	1.28	0.76–2.15	16.7	0.005	2.05	1.20–3.49	2.4	0.847	0.90	0.25–2.96
	RU	16.3	0.102	1.43	0.91–2.25	11.3	0.622	0.89	0.54–1.46	8.8	0.945	0.98	0.55–1.73	5.8	0.029	2.27	1.00–5.15
Insect	Without atopy	39.2	—	1	—	22.3	—	1	—	11.5	—	1	—	0.0	—	1	—
	AD	71.4	0.001	3.88	1.63–9.40	60.0	< 0.001	5.23	2.25–12.28	45.7	< 0.001	6.49	2.60–16.30	0.0	ND	ND	ND
	RU	78.0	< 0.001	5.50	2.47–12.4	40.0	0.014	2.32	1.11–4.87	44.0	< 0.001	6.05	2.68–13.80	10.0	ND	ND	ND

*Percentage of positive reactions = (No. of positive reactions for all horses/No. of possible positive reactions) $\times$ 100.

OR = Odds ratio. CI = 95% Confidence interval. — = Not applicable. ND = Not determined, because a value of 0 was included in the 2 $\times$ 2 table.

Table 8—Scores* for histopathologic findings in lung parenchyma of horses without atopy

Category	Mean	SD
Bronchiolar neutrophils	0.1	0.1
Bronchiolar eosinophils	0	0
Bronchiolar epithelial hyperplasia	0.1	0.2
Bronchiolar luminal exudate	0	0
Peribronchiolar mast cells	0	0
Peribronchiolar lymphoplasmacytic infiltrations	0.1	0.2
Peribronchiolar fibrosis	0	0
Goblet cell metaplasia	0	0
Alveolar fibrosis or emphysema	0	0
Pneumocyte II hyperplasia	0	0
Total†	0.3	0.5

*Scores were determined on the basis of a 2-point scale. †Sum of scores for all categories.

lack of disease in horses without atopy. Examination of lung tissue in all horses without atopy did not reveal gross abnormalities. Histologic scores of parenchyma of lung specimens from the left dorsal apical region of horses without atopy all were ≤ 0.2 (Table 8).

Discussion

In the study reported here, horses with AD or RU had a significantly greater mean number of positive reactions for immediate (30 minutes), late-phase (4 and 6 hours), and delayed (24 hours) reactions to intradermally injected environmental allergens, compared with reactions in horses without atopy. All horses in our study had the greatest number of positive reactions at 30 minutes. This finding is similar to that of Evans et al¹⁶ who reported that clinically normal horses, horses with RU, and horses with COPD had the greatest number of reactions 15 to 30 minutes after injection during IDT. Our finding that the mean number of positive reactions for horses with RU was greater than that for horses without atopy at all observation times also is similar to the findings in that other study.¹⁶

The significantly greater mean number of positive immediate, late-phase, and delayed reactions in our horses with AD or RU, compared with the number for our horses without atopy, indicates that results of IDT can provide useful confirmatory evidence for the diagnosis and treatment of horses with AD or allergic RU. Positive reactions in our horses without atopy is consistent with reported positive reactions for IDT conducted in clinically normal horses,^{7,15} dogs,³² and humans.³³ Clinical importance of positive reactions for a specific horse should be interpreted with caution, because subclinical sensitization in our horses without atopy underscores the fact that IDT does not necessarily distinguish a state of clinical allergy from that of subclinical sensitization.

Differences in the pattern of reactivity (mean number of positive reactions, reactivity to specific groups of allergens, or reactivity at differing observation times) can be helpful in distinguishing clinical allergy from subclinical hypersensitivity and were seen in both groups of allergic horses reported in this study. The *P* values and odds ratios for data at 30 minutes after injection of allergens indicated that horses with AD have a significantly greater number of positive reac-

tions during the immediate phase to all allergen groups, compared with results for horses without atopy. This also is true for the comparison of horses with RU and horses without atopy, except for the results for mold allergens. This evidence strongly supports the pathogenic role of allergen-specific IgE in horses with AD or RU. More specifically, in historically and clinically characterized horses with AD or RU, positive reactions at 30 minutes in $\geq 25\%$ of allergens tested (≥ 18 positive reactions for 73 allergens evaluated in our study) were strongly suggestive of clinically relevant allergic hypersensitivity. In our study, 4 of 7 horses with AD and 6 of 10 horses with RU had positive reactions to $\geq 25\%$ of allergens at 30 minutes.

Interestingly, horses with AD had significantly greater odds of having positive LPR in all aeroallergen groups than did horses without atopy, except for mold allergens at 4 hours. In humans, it is recognized that immediate hypersensitivity reactions are frequently followed by LPR. The T lymphocytes, predominantly T-helper 2 cells, are believed to play a major role in the accumulation and activation of eosinophils and, possibly, basophils at the site of LPR.³⁴ The exact relationship between an immediate reaction and LPR remains to be defined.³⁴ However, it is believed that chronic allergic inflammation in the skin and lungs may be a manifestation of persistent LPR.³⁴ Investigators have examined biopsy specimens from sites of positive reactions at 4 to 6 hours for IDT in horses affected with allergic COPD, and they reported histopathologic findings compatible with LPR of humans.^{7,35} In our horses, the time of development of these reactions in horses with AD was similar to that of humans and may represent a common pathway in the pathogenesis of AD in these species.

The pathogenic importance of the greater number of delayed reactions in horses with RU, compared with that in horses without atopy or horses with AD, remains to be clarified. Because most of the delayed reactions in horses with RU were preceded by immediate reactions or LPR, it is likely that the delayed reaction was an extension of the altered state of immune reactivity associated with IgE-mediated hypersensitivity. However, 10 of 38 (26%) were not preceded by an immediate reaction or LPR. It is possible that an immediate reaction or LPR did develop but was scored as negative or was not grossly visible.

The high percentage of positive reactions at 4 hours that were not preceded by immediate reactions in horses without atopy (38%), horses with AD (26%), and horses with RU (18%) indicates that examination of the injection site at 4 hours after administration of allergens for IDT may be important for determining the full spectrum of allergen hypersensitivity in a particular horse. In the 7 horses with AD reported here, only 11 reactions at 6 hours and 2 reactions at 24 hours were not preceded by a positive reaction at an earlier observation point. These account for 8 of 421 ($< 1\%$) of all positive reactions. Similarly, in the 10 horses with RU reported here, only 6 reactions at 6 hours and 10 reactions at 24 hours were not preceded by a positive reaction at an earlier observation point. These account for 16 of 540 ($< 3\%$) of all positive reactions. Analysis

of these results suggests that observation for reactions 30 minutes and 4 hours after injection of allergens provides adequate information of allergen-specific hypersensitivity.

Black ant extract, grain smut mix, and grain mill dust mix consistently had the highest overall reactivity at all time points in all 3 groups of horses. The high prevalence of positive reactions to these allergens (45 to 51% of all horses) raises the possibility that some or all of these reactions represent false-positive irritant reactions. It has been suggested that any allergen that causes a positive test result in > 25% of clinically normal animals is potentially irritating and capable of causing positive test results that are not truly reflective of allergen-specific IgE-mediated hypersensitivities.³⁵ Thus, the threshold concentration of an allergen is defined as the highest concentration or largest amount of allergen administered that will produce a positive result in < 25% of clinically normal animals.³⁵ Concentration for the black ant extract (1,000 PNU/ml) is the concentration commonly used in dogs.³⁵ However, > 25% of the 22 horses without atopy had positive results to this concentration of black ant extract, and a lower concentration may be more appropriate for use in horses.

Similarly, the high prevalence of positive reactions to grain smut mix and grain mill dust mix in the study reported here and the study of Evans et al¹⁶ raises the possibility of false-positive reactions with these extracts. Furthermore, grain mill dust mix is a collection of dust from a grain mill.^m Therefore, the extract is a mixture of barley, corn, oat, and wheat grains as well as danders, mold spores, other insect allergens, pollens, and nonallergenic proteins. This extract is standardized (wt:vol) such that a specific weight of defatted allergen is diluted in a specified volume of fluid, and the relative amount of each particular allergen may vary. Consequently, reactivity to grain mill dust mix is nonspecific for any particular allergen. Thus, the diagnostic value of grain mill dust mix and grain mill smut remains to be elucidated. Despite these reservations, we cannot rule out the possibility that reactions to these allergens in the horses of this study may represent clinical and subclinical hypersensitivity.

Analysis of results of the study reported here provide strong evidence of IgE-mediated hypersensitivity in the pathogenesis of horses with AD or RU. However, the relative clinical importance of hypersensitivity to a particular allergen and selection of allergens for inclusion in an immunotherapeutic vaccine still is dependent on the medical history, clinical signs, and probable exposure to allergens for each horse.

^aCoulter Counter S plus IV, Coulter Corp, Hialeah, Fla.

^bHitachi 911, Boehringer Mannheim, Indianapolis, Ind.

^cXylazine HCl, Butler Co, Dublin, Ohio.

^dBivona, Jorgensen Labs Inc, Loveland, Colo.

^eCytospin Shandon Elliot, Shandon Southern Instruments, Camberly, England.

^fHistatrol, Center Laboratories Inc, Port Washington, NY.

^gSterile diluent for allergenic extracts, Greer Laboratories Inc, Lenoir, NC.

^hGreer Laboratories Inc, Lenoir, NC.

ⁱAngiocath, Becton-Dickinson Co, Sandy, Utah.

^jBeuthanasia-D special, Schering Plough Animal Health, Kenilworth, NJ.

^kSAS, version 6.12, SAS Institute Inc, Cary, NC.

^lStatCalc, Epi Info Inc. Available at: <http://www.cdc.gov/epo/epi/intro/manual/mantoc.htm>. Accessed November 1999.

^mBarbara Vignola, Division of Veterinary Products, Greer Laboratories Inc, Lenoir, NC: Personal communication, 1999.

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