Hypersensitivity disorders in horses are commonly associated with the development of skin disease. These allergic diseases, such as IBH, atopic dermatitis, and urticaria, cause variable signs in horses, including pruritus, signs of pain, and changes to the coat.1 Horses with severe disease can lose body condition and may have substantial decreases in athletic performance.

The prevalence of most allergic skin diseases of horses is unknown, except for IBH, which has a reported prevalence of 11.6% to 71.4%.2-6 Research in the field of allergic skin disease in horses has been hampered, at least in part, by the need for a practical model of the disease. In the ideal situation, horses with spontaneous allergic skin disease would be used for study. However, it is difficult to develop a herd of sensitized horses with allergic skin disease, although lymphocytes were not increased; thus, ID injection of anti-IgE in horses may be of use for evaluating allergic skin diseases of horses. (Am J Vet Res 2014;75:633–641)
the additional variables associated with administration of allergen extracts. Researchers have effectively developed models of cutaneous allergic inflammation in other species, including dogs \cite{10,16} and humans. \cite{10,16}

Briefly, allergic skin disease is often mediated by type I hypersensitivity reactions. These reactions (immediate and late-phase hypersensitivity) are initiated by antigen exposure. On exposure, naïve antigen-specific T cells are induced to differentiate into \( T_2 \) phenotype cells, which then activate B cells. The B cells undergo class switching and produce IgE, which binds to the high-affinity receptor for IgE (ie, FcεRI) on the surface of mast cells. During subsequent exposure to the same antigen, the surface-bound IgE is cross-linked and the mast cells become activated. Mast cells release mediators (histamine, cytokines, and leukotrienes) that lead to the development of immediate and late-phase hypersensitivity reactions. Late-phase reactions are characterized by an influx of inflammatory cells, including neutrophils, eosinophils, basophils and T cells, 6 to 48 hours after challenge exposure to the allergen. \cite{8,17,18}

Atopic dermatitis \cite{1} and IBH \cite{1,19} in horses involve type I hypersensitivity reactions. The involvement of IgE, specifically, has been suggested \cite{7,20} and established \cite{21,22} in allergic skin disease of horses. Horses affected by IBH have increased expression of both IgE protein and mRNA in dermal cells of affected skin, compared with results for unaffected skin of clinically normal horses, especially in the acute phase. \cite{23} Examination of skin biopsy specimens obtained from horses with urticaria also revealed that there were more IgE-bearing cells in the superficial dermis, compared with the number of those cells in skin biopsy specimens obtained from clinically normal horses. \cite{22}

Type I hypersensitivity reactions can be simulated by ID injection of anti-IgE antibodies, \cite{23} which function to cross-link surface IgE and mimic the effects of an allergen. This reaction has been used in humans to evaluate the effects of drugs on IgE-mediated reactions. \cite{10,16} In dogs, a model of allergic dermatitis has been successfully developed by the use of anti-IgE. \cite{8,11} To our knowledge, such a model of allergic dermatitis in horses has not been developed.

The objective of the study reported here was to develop a method that would mimic allergic dermatitis in horses. The hypothesis was that ID injection of anti-IgE in clinically normal horses would result in macroscopic and microscopic reactions similar to those of horses with allergic skin disease. Additionally, these reactions would be comparable to those observed in dogs after injection of anti-IgE.

**Materials and Methods**

**Animals**—Six horses (3 geldings and 3 mares; 4 to 8 years old) were used for the study. Horses were chosen on the basis of a lack of evidence of current or prior cutaneous or systemic disease (including allergic skin disease) as determined during physical examinations. All horses were vaccinated against eastern equine encephalomyelitis, western equine encephalomyelitis, West Nile virus encephalomyelitis, tetanus, and influenza and dewormed with a product containing ivermectin or moxidectin (200 μg/kg, PO, q 6 mo) as part of routine herd care. No medications were administered for at least 14 days prior to the study. All experimental protocols were approved by the Louisiana State University Institutional Animal Care and Use Committee.

**Generation of IgE-mediated reactions**—Hair was shaved from the lateral cervical area of each horse on day 0 in preparation for injections in this location. Horses were sedated by IV administration of xylazine hydrochloride (0.4 mg/kg). All horses were injected ID (0.1 mL/injection) at 1 site with PBS solution, at 4 sites with protein G affinity-purified anti-IgE \cite{24} (prepared by one of the authors [BH]) diluted to 0.1 mg/mL in PBS solution, and at 4 sites with rabbit IgG \cite{25} diluted to 0.1 mg/mL in PBS solution (time of injections was designated as time 0). Anti-canine IgE was used because anti-equine IgE was not available. Furthermore, preliminary experiments conducted by the authors (data not shown) indicated the ability of anti-canine IgE to induce development of gross and microscopic immediate and late-phase reactions in the skin of clinically normal horses. In those preliminary experiments, ID injection of the same anti-IgE product (at a lower dose) into 4 horses resulted in wheals and histopathologic changes consistent with those observed in horses with allergic skin disease.

Rabbit antisera specific for canine IgE was generated by injection of canine monoclonal IgE \cite{26} produced by a canine \times mouse heterohybridoma. \cite{27} Antisera were adsorbed with canine IgG linked to beads to remove antibodies cross-reactive with IgG.

**Grading of macroscopic reactions**—Sites of anti-IgE and IgG injections were examined 20 minutes and 6, 24, and 48 hours after injection for the development of cutaneous swelling, erythema, or induration. Sites of injection with PBS solution were measured 20 minutes after injection. Diameter of the cutaneous reactions (ie, wheals) was measured in 2 perpendicular directions, and surface area of each reaction was calculated. Dermal thickness of the injected skin was measured with calipers.

**Specimen collection and processing**—Horses were sedated with xylazine, and areas for acquisition of biopsy specimens were injected SC with 0.5 mL of 2% lidocaine hydrochloride. \cite{28} One 8-mm punch biopsy specimen of clinically normal, noninjected (ie, no ID injections) skin on the lateral aspect of the neck was collected at the time of ID injections. Samples were collected at anti-IgE and IgG injection sites 20 minutes and 6, 24, and 48 hours after injection. Each sample was bisected immediately after collection. One half was placed in neutral-buffered 10% formalin for routine processing in paraffin. Fixation time differed but was at least 72 hours. The other half was placed in cryopreservation embedding medium, \cite{29} frozen in isopentane cooled in liquid nitrogen, and stored at –80°C until cryosectioning.

**Histologic examination**—Tissue sections (thickness of 5 μm) were cut from paraffin-embedded blocks, stained with H&E, and used for histologic examination, cell enumeration, and pattern analysis. Luna stain was used to facilitate evaluation of eosinophils. \cite{30} A low-
pH (pH, 2.5) toluidine blue stain was used to facilitate evaluation of mast cells.

**Immunophenotypical analysis of cutaneous mononuclear cells**—A modification of a 3-step streptavidin-labeled method was used to characterize the mononuclear cell infiltrate. Briefly, 6-µm-thick cryosections were air-dried and then immersed in acetone for fixation. Samples were rinsed with PBS solution containing 0.5% Tween 20 and then blocked with 1% fetal calf serum. Blocking sera were drained, and mouse-origin monoclonal antibodies specific for equine CD4 or CD8 β chain were diluted 1:70 in PBS solution and applied to the slides. After the slides were rinsed with PBS solution containing 0.5% Tween 20, endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide in PBS solution. Biotinylated horse-origin anti-mouse IgG diluted 1:400 in PBS solution was applied, which was followed by application of horseradish peroxidase–conjugated streptavidin diluted 1:400 in PBS solution. Samples were rinsed between steps with PBS solution containing 0.5% Tween 20.

Amino-9-ethyl carbazole was applied as a chromogen, which was followed by application of hematoxylin counterstain.

For CD3 immunohistochemical analysis, slides were deparaffinized by immersion in xylene (3 times, 5 min/immersion, with fresh xylene used for each immersion). Samples were then rehydrated in 100% alcohol (3 times, 5 min/immersion, with fresh alcohol used for each immersion) and then immersed in 95% alcohol for 5 minutes; rehydrated samples were rinsed 3 times with deionized water. Antigen retrieval was performed by immersion in 10mM citrate buffer (pH, 6.0) for 10 minutes at 125° to 127°C, and slides then were allowed to cool for 20 minutes before being rinsed 3 times (each rinse consisted of deionized water followed by Tris-buffer with 1% Tween 20 [pH, 7.6]). The remainder of the procedure was performed in an autostainer. Endogenous peroxide activity was blocked by incubation with 3% hydrogen peroxide for 3 minutes, which was followed by rinsing with Tris-buffer with 1% Tween 20. Binding of nonspecific antibodies was blocked by incubation with normal goat serum for 30 minutes followed by incubation with primary antibody (rabbit anti-human CD3 diluted 1:400) for 30 minutes, which was followed by rinsing with Tris-buffer with 1% Tween 20. Samples then were incubated with secondary antibody (biotinylated anti-rabbit secondary antibody) for 30 minutes and rinsed with Tris-buffer with 1% Tween 20. Tissue samples were exposed to the detection reagent avidin-biotinylated enzyme complex for 30 minutes, which was followed by rinsing with Tris-buffer with 1% Tween 20 and application of membrane stain and by additional rinsing with Tris-buffer with 1% Tween 20 followed by deionized water. Samples were counterstained by incubation with Mayer’s hematoxylin (Lillie’s modification) for 5 minutes, rinsed again with Tris-buffer with 1% Tween 20 followed by deionized water, and removed from the stainer. Slides were dried in an oven at 60°C for 30 min-

**Figure 1**—Photomicrograph of a section of skin indicating dermal depths used to evaluate inflammatory cell location in horses. Bar = 200 µm.

**Figure 2**—Mean ± SE surface area (A) and skin thickness (B) of local urticarial reactions after ID injection of anti-IgE at 4 locations (black bars), rabbit IgG at 4 locations (light gray bars), and PBS solution at 1 location (dark gray bars) in 6 clinically normal horses. Skin samples after injection of PBS solution were measured only at 20 minutes. *Within a time period, value differs significantly (P < 0.001) from the value for the IgG injection. †Value differs significantly (P < 0.05) from the value for the PBS solution injection at 20 minutes. ‡Within a treatment, value differs significantly (P < 0.05) from the value at the preceding time point.
utes and then were allowed to cool for 5 to 10 minutes. Slides were rinsed in xylene (3 times, 3 min/rinse, with fresh xylene used for each rinse), and a coverslip was then applied to each slide.

**Enumeration of dermal cells**—The total number of cells/mm² in the dermis was obtained for all cell types by counting the number of cells in 16 fields (0.25 X 0.25 mm) of the dermis (excluding epithelial cells and the adnexa). For slides stained with H&E, Luna stain, and toluidine blue, the 16 fields were allocated evenly among 4 depths of dermis: superficial dermal, superficial follicular, deep follicular, and deep follicular to adnexal (Figure 1). Numbers of nucleated cells, granulocytes, and mononuclear cells were counted on H&E-stained slides. Eosinophils were counted on slides stained with Luna stain. Total neutrophil count was obtained by determining the

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**Figure 3**—Mean ± SE number of mast cells (A) and degranulated mast cells (B) in skin samples obtained after ID injection of anti-IgE (black bars) and rabbit IgG (gray bars) or in noninjected skin (white bars) of 6 clinically normal horses. Noninjected skin samples were collected only before injection (time 0). The scale for the y-axis differs between panels. Notice that ID injection of anti-IgE caused degranulation of mast cells.

**Figure 4**—Mean ± SE number of inflammatory cells (A), number of eosinophils (B), dermal depth of eosinophils (C), and number of neutrophils (D) in skin samples obtained after ID injection of anti-IgE and rabbit IgG or in noninjected skin of 6 clinically normal horses. The scale on the y-axis differs among panels. Notice that ID injection of anti-IgE caused a late-phase cellular response. *Value differs significantly (P < 0.05) from the value for the IgG injection within a time point (panels A, B, and D) or within a dermis depth (panel C). †Value differs significantly (P < 0.05) from the value for noninjected skin. ‡Within a treatment, value differs significantly (P < 0.05) from the value at the preceding time point. See Figure 3 for remainder of key.
difference between the number of eosinophils/mm² and number of granulocytes/mm². Slides stained with toluidine blue were evaluated to determine the number of intact mast cells as well as the number of degranulated mast cells. The CD3⁺, CD4⁺, and CD8⁺ cells were counted in 16 fields (0.25 X 0.25 mm) beginning in the superficial dermis (excluding endothelial cells and adnexa) and extending into deeper dermal layers.

Statistical analysis—Data analysis was performed with statistical software. A repeated-measures ANOVA with a factorial arrangement of treatments was conducted for dermal cell counts and injection site measurements. Fixed effects included dermal depth (when applicable), time, treatment, and the interactions. Animal was included as a random effect. When significant (P ≤ 0.05) differences were detected, post hoc comparisons were performed with pairwise t tests of least squares means.

Results

Macroscopic reactions—Intradermal injection of anti-IgE induced erythematous and indurated local urticarial reactions within 20 minutes after injection in all horses. The surface areas of those reactions were significantly larger than the surface areas of reactions at 20 minutes and 6 and 24 hours after injection of IgG (Figure 2). The surface area of dermal induration after anti-IgE injection significantly increased from 20 minutes to 6 hours and from 6 to 24 hours. Surface area at sites of anti-IgE injection was significantly larger than the surface area at sites of PBS solution injection at ≥ 20 minutes, except at 48 hours after injection. Surface area at sites of IgG injection, when measurable, never differed significantly from the surface area at sites of PBS solution injection at 20 minutes. There were no measurable wheals or induration at sites of IgG injection at 24 or 48 hours or at sites of anti-IgE injection at 48 hours.

The anti-IgE injection sites were significantly thicker than the IgG injection sites at 20 minutes and 6 and 24 hours after injection (Figure 2). Dermal thickness at sites of anti-IgE injection was greatest 20 minutes after injection and decreased significantly at all time points, compared with the previous values (ie, from 20 minutes to 6 hours, from 6 to 24 hours, and from 24 to 48 hours). Thickness at sites of anti-IgE injection was significantly greater than thickness at sites of PBS solution injection at 20 minutes and 6 hours but was significantly less than thickness at sites of PBS solution injection at 48 hours. Thickness at sites of IgG injection was less than the thickness at sites of PBS solution injection at 24 and 48 hours.

Immediate reactions—No significant difference in the total number of mast cells was found between treatments (Figure 3). The mean number of degranulated mast cells was significantly (P < 0.001) higher for anti-IgE injections than for IgG injections, but a treatment-by-time interaction was not detected.
Late-phase reactions—The total number of inflammatory cells increased up to 24 hours after anti-IgE injection, compared with the number of cells at time 0, and there were significantly more inflammatory cells at sites of anti-IgE injection than at sites of IgG injection (Figure 4). By 48 hours, the total number of inflammatory cells had decreased at sites of anti-IgE injection and did not differ significantly, compared with inflammatory cell counts in noninjected skin at time 0.

Examination of slides stained with Luna stain revealed a significant increase in the number of eosinophils at sites of anti-IgE injection from 20 minutes to 6 hours and from 6 to 24 hours (Figure 4); eosinophil counts significantly decreased from 24 to 48 hours. There were significantly more eosinophils at sites of anti-IgE injection than at sites of IgG injection at 6 hours (P < 0.002), 24 hours (P < 0.001), and 48 hours (P = 0.001). Sites of anti-IgE injection had significantly more eosinophils at 6 hours (P = 0.001), 24 hours (P < 0.001), and 48 hours (P = 0.001), compared with the number of eosinophils in noninjected skin at time 0. Eosinophil counts did not differ significantly between sites of IgG injection at any time point and noninjected skin at time 0.

A significant treatment-by-depth effect was detected for eosinophils. Eosinophil counts were significantly higher in the superficial follicular (P = 0.001), deep follicular (P < 0.001), and deep follicular to adnexal (P < 0.001) dermis for sites of anti-IgE injection, compared with counts at those dermal depths for sites of IgG injection (Figure 4). Eosinophil counts in the superficial dermis did not differ between treatments.

Neutrophils were significantly increased at sites of anti-IgE injections from 20 minutes to 6 hours. Neutrophil counts decreased significantly from 6 to 24 hours and 24 to 48 hours (Figure 4). There were significantly more neutrophils at 6 hours at sites of anti-IgE injection than at sites of IgG injection. Significantly more neutrophils were detected for anti-IgE injection at 6 and 24 hours than for noninjected skin at time 0. Neutrophil counts for IgG injection at any time point did not differ significantly from those of noninjected skin at time 0.

Immunophenotypical analysis of mononuclear cells—No significant difference was detected for CD3+, CD4+, or CD8+ cell counts in the dermis at any time point between sites of anti-IgE injection and IgG injection (Figure 5).

Discussion

Analysis of results of the present study confirmed that ID injection of protein G affinity-purified rabbit-origin polyclonal IgG specific for canine IgE induced many macroscopic and microscopic changes in clinically normal horses comparable to those seen in humans and dogs in similar studies. Anti-equine IgE was not available for use in the present study but would have been expected to yield similar results. The reported protein sequence of the epsilon chain of canine IgE is approximately 65% to 67% identical with that of equine IgE,29,30 which would suggest sufficient similarity to permit cross-species use. This assumption is supported by the fact that some commercially available monoclonal anti-canine IgE antibodies can cross-reactively bind to equine IgE. In the experience of one of the authors (BH), ID injections of both rabbit-origin polyclonal anti-canine IgE and mouse monoclonal anti-IgE were able to induce immediate wheal-and-flare responses in the skin of clinically normal horses. In addition, a preliminary unpublished study conducted by one of the authors (MCW) indicated that ID injection of the same rabbit-origin polyclonal anti-IgE product, but at a lower dose, in 4 horses resulted in grossly measurable wheals consistent with those seen following allergen injection in allergic horses. Furthermore, histologic examination of those injection sites at various time points revealed mast cell degranulation and eosinophil recruitment. However, the authors acknowledge that the use of anti-canine IgE in horses is less than ideal and may be a contentious issue.

Intradermal injection of cross-linking anti-IgE induced an immediate wheal response at 20 minutes, which continued to increase in surface area through 24 hours (Figure 2). In a similar study9 in dogs, a significantly larger wheal was evident for anti-IgE injection than for IgG injection at 20 minutes and 6 hours but not at 24 or 48 hours. In another study9 in dogs, urticaria was observed through 24 hours, though lesions decreased in size from the initial examination until 24 hours. Thus, it appears that horses may have a more prolonged macroscopic late-phase reaction than do dogs.

Intradermal injection of PBS solution and IgG also induced measurable distention of the skin, but these areas were significantly smaller and less indurated than were anti-IgE wheals. Additionally, there were no wheals associated with IgG injection by 24 hours. Intradermal injection of any substance will cause transient distention of the superficial dermis, but this distention will dissipate with time as the substance is absorbed. This likely accounted for the initial injection site measurements obtained for injections of IgG and PBS solution.

The use of stereological methods for counting of cells was considered during the design of the present study. However, these programs were not used for several reasons. The first reason was the complex structure of the superficial and middle dermis. In these areas, there are numerous nondermal structures, including the papillary epidermis, hair follicles, sebaceous glands, muscles, blood vessels, and nerves. To consistently count cells in an area of dermis, these structures need to be excluded from that area, which is easiest for a human during manual counting. Additionally, these other structures and their associated cell types (keratinocytes, sebocytes, and endothelial cells) can make it difficult to identify specific cell types of interest (mononuclear cells and granulocytes). For these reasons, we chose to use a manual method for cell counting in this study.

Although ID injection of anti-IgE yielded tissue with significantly more degranulated mast cells than in noninjected tissue at time 0 and IgG-injected tissues at any time point, a treatment-by-time interaction was not apparent. One possible reason for a lack of significant differences with regard to degranulated mast cells was the difficulty in visually identifying them once they had...
and thus would not have been expected to have a local a preexisting hypersensitivity to the anti-IgE antibody cause the horses in the study were unlikely to have had sinophil recruitment was likely attributable to chemo- in the skin of horses with IBH following injection with Culicoides antigen. These late-phase changes were not seen in the present study. However, the investigators in that study32 only evaluated horses with signs of IBH, and none of the horses in the present study had signs of IBH. It is possible that if the present study were repeated with allergic horses injected ID with a specific antigen, the results would be more similar to those seen in that other study.32 Additionally, it must be mentioned that the investigators in that previous study32 evaluated the response to whole-body Culicoides extract, which may have included proteases and other irritants. That extract failed to induce a gross wheal response in non-allergic ponies,34 and the Culicoides injection sites were not histologically evaluated in those animals. Thus, it is difficult to ascertain whether the lymphocyte responses truly represent an antigen-specific phenomenon.

The CD4+ cell counts in the present study were not consistent with findings in other studies.32,35 Investigators in 1 study35 on Icelandic horses with IBH found an increase in CD4+ cells in skin samples of affected horses. Although the cause of the lack of increase of CD4+ cells in the present study is not known, it may have been partially attributable to the difficulty in performing immunohistochemical analysis on frozen tissues. In general, the architecture of the skin in these samples was not maintained well, and the samples were more difficult to stain than were paraffin-embedded tissues. In addition, the use of anti-canine IgE (rather than anti-equine IgE) may have led to the difference in the depth of dermis at which eosinophils are found in horses with naturally occurring allergic skin disease.

Quantitation of CD3+, CD4+, and CD8+ cells over time in the present study did not reveal significant differences between anti-IgE and IgG injections. The CD3+ stained slides were assessed for formalin-fixed paraffin-embedded tissue and were evaluated separately from the CD4+ and CD8+ cells counts, which were obtained from frozen tissue. This may have accounted for the decreased number of CD3+ cells, compared with the sum of CD4+ and CD8+ cell counts (because all of these cells should also have been CD3+ cells). The anti-IgE injection resulted in increased numbers of CD3+ cells, compared with the number of CD3+ cells after IgG injection, from 20 minutes to 24 hours with a subsequent decrease in cell counts at 48 hours, but these values did not differ significantly (Figure 5). This pattern was similar to that for a previous study in dogs in which CD3+ cells increased by 6 hours and peaked 24 hours after anti-IgE injection. Another study46 conducted to evaluate late-phase reactions after ID injection of anti-IgE in dogs found that dermal counts of CD3+ cells increased from 6 to 48 hours after injection. Investigators evaluated the skin lymphocyte populations in horses with IBH32 and found that there were an increased number of CD3+ lymphocytes (many of which were also CD4+) in the skin of horses with IBH following injection with Culicoides antigen. The CD4+ cell counts in the present study were not consistent with findings in other studies.32,35 Investigators in 1 study35 on Icelandic horses with IBH found an increase in CD4+ cells in skin samples of affected horses. Although the cause of the lack of increase of CD4+ cells in the present study is not known, it may have been partially attributable to the difficulty in performing immunohistochemical analysis on frozen tissues. In general, the architecture of the skin in these samples was not maintained well, and the samples were more difficult to stain than were paraffin-embedded tissues. In addition, the use of anti-canine IgE (rather than anti-equine IgE) may have led to the difference in the depth of dermis at which eosinophils are found in horses with naturally occurring allergic skin disease.

Historically, eosinophilic dermatitis has been con- sistently seen with acute allergic skin disease in horses; however, the depth of eosinophilic infiltration was not described or the relative numbers of eosinophils at various depths were not compared.1,12,21,31,33 In the present study, more eosinophils were found in the deep layer of dermis in anti-IgE injected skin. Investigators of a previous study24 conducted to evaluate effects of ID injection of anti-IgE in dogs described cellular counts only in the most superficial dermis. Results of the study reported here indicated that there were no significant differences in eosinophil numbers in the superficial dermis between anti-IgE and IgG injections (Figure 4). However, eosinophil numbers were increased at the depth of the follicle and deeper dermis after anti-IgE injection, compared with eosinophil numbers after IgG injection. Had this study not evaluated depth as a factor, an important part of the late-phase reaction (eosin- ophil recruitment) might have been missed. The reason for the increased eosinophil recruitment to the deeper dermis is not known. Further studies will be needed to evaluate the depth of dermis at which eosinophils are found in horses with naturally occurring allergic skin disease.

AJVR, Vol 75, No. 7, July 2014

639

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The CD8+ cells have been evaluated less commonly than have CD3+ and CD4+ cells in horse skin. In the present study, CD8+ cells increased in anti-IgE injected skin from 20 minutes to 24 hours and then decreased at 48 hours (similar to the CD3+ cell counts). However, the numbers did not differ significantly from those after IgG injection. In 1 study,23 investigators found significantly more dermal CD4+ cells, but not more dermal CD8+ cells, in lesional skin from horses with IBH, compared with skin from healthy horses.

Horses in the study reported here developed gross and histologic reactions following ID injection of anti-IgE. Therefore, IgE must have been present at some degree at the sites of injection prior to the injection of anti-IgE. Studies7,36,37 have revealed that horses that do not have clinical allergic skin disease can still be sensitized to allergens and have positive reactions to ID injections. In contrast to specific allergens, anti-IgE has been used to desensitize horses and have positive reactions to ID injections.14,35,36,38,39 In horses have found that IgE on mast cells cross-links all IgE, regardless of allergen specificity.21

The study reported here confirmed that injection of anti-IgE does not necessarily reflect an allergy or parasitism. As previously mentioned, ID injection of anti-IgE has been used in humans and dogs to mimic allergic skin disease and allow evaluation of the effects of various pharmaceutical agents on immediate and late-phase inflammatory reactions.9,10,12–16,40 The study reported here confirmed that injection of anti-IgE induces a dermal inflammatory response with many similarities to those seen in horses with spontaneous allergic skin disease. These similarities were particularly marked for eosinophils and neutrophils, although the lymphocyte response generated in this study was of lower magnitude than would be expected. In the future, controlled studies could be performed by use of these methods to enable evaluation of the efficacy of various treatment modalities and their effects on macroscopic and microscopic reactions for allergic skin disease in horses without the need to maintain a herd of allergic horses.

a. Lloyd Laboratories, Decatur, Ill.
c. Bethyl Labs, Montgomery, Tex.
d. Sparhawk Laboratories, Lenexa, Kan.
e. tissue-Tek OCT Compound, Sakura Finetek USA Inc, Torrance, Calif.
f. Amresco LLC, Solon, Ohio.
g. VMRD, Pullman, Wash.
h. Vector Laboratories, Burlingame, Calif.
i. Invitrogen, Life Technologies Inc, Grand Island, NY.
j. AEC, BioGenex, Fremont, Calif.
k. Dako North America Inc, Carpenteria, Calif.
l. DAKO AutoStainer LINK 48, Dako North America Inc, Carpenteria, Calif.
m. Vector Laboratories, Burlingame, Calif.
n. DAKO A0452, Dako North America Inc, Carpenteria, Calif.
.o. Vector BA 1000, Vector Laboratories, Burlingame, Calif.
p. Vectastain ABC rabbit IgG kit, Vector PK 6102, Vector Laboratories, Burlingame, Calif.
q. Vector Sk-4800, Vector Laboratories, Burlingame, Calif.
s. MCA1896, ABD Serotec, Raleigh, NC.


