



Equine Skin: Structure, Immunologic Function, and Methods of Diagnosing Disease

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ABSTRACT:

A horse's skin provides an anatomic and physiologic barrier between the external and internal environment; aids in thermoregulation; perceives heat, cold, pain, pruritus, touch, and pressure; and provides pigmentation. Despite frequently encountered skin disorders in horses, dermatology is inadequately investigated in equine medicine. Therefore, much information in this article has been extrapolated from other species, including humans, dogs, and cats. This article describes the normal anatomy of the equine skin, immunologic cells related to the integument, and ancillary diagnostic tests available for several allergic skin disorders in horses.

Dermatologic disorders are often encountered by equine practitioners and require a thorough evaluation, including the duration, distribution, and description of lesions and associated clinical signs. This article reviews the anatomic features of equine skin and alternative diagnostic methods that may provide practitioners with additional tools to evaluate equine skin disorders.

GROSS AND HISTOLOGIC ANATOMY OF EQUINE SKIN

The skin comprises the epidermis and underlying dermis. A horse's skin provides an anatomic and physiologic barrier between the external and internal environment; aids in thermoregulation; perceives

heat, cold, pain, pruritus, touch, and pressure; and provides pigmentation.^{1,2} The epidermis, with an average thickness of 0.053 mm, contains multiple layers of cells and is the outermost nonvascular layer of the skin.³ The cell layers of the epidermis originate from a basal layer and are modified as they move superficially. The epidermis imparts pigmentation, immunologic regulation, and touch perception. In comparison, the dermis is much thicker; it supports the epidermis and provides flexibility to the skin through its composition of elastin and collagen. The thickness of the dermal layer varies throughout a horse's body, depending on the region of the body, and ranges from 1 to 6 mm (average: 3.8 mm).⁴ The thickest areas are located on the dorsum (i.e., head, mane, back, croup, tail), whereas the thinnest regions are on the ventrum (i.e., udder, medial thigh, external

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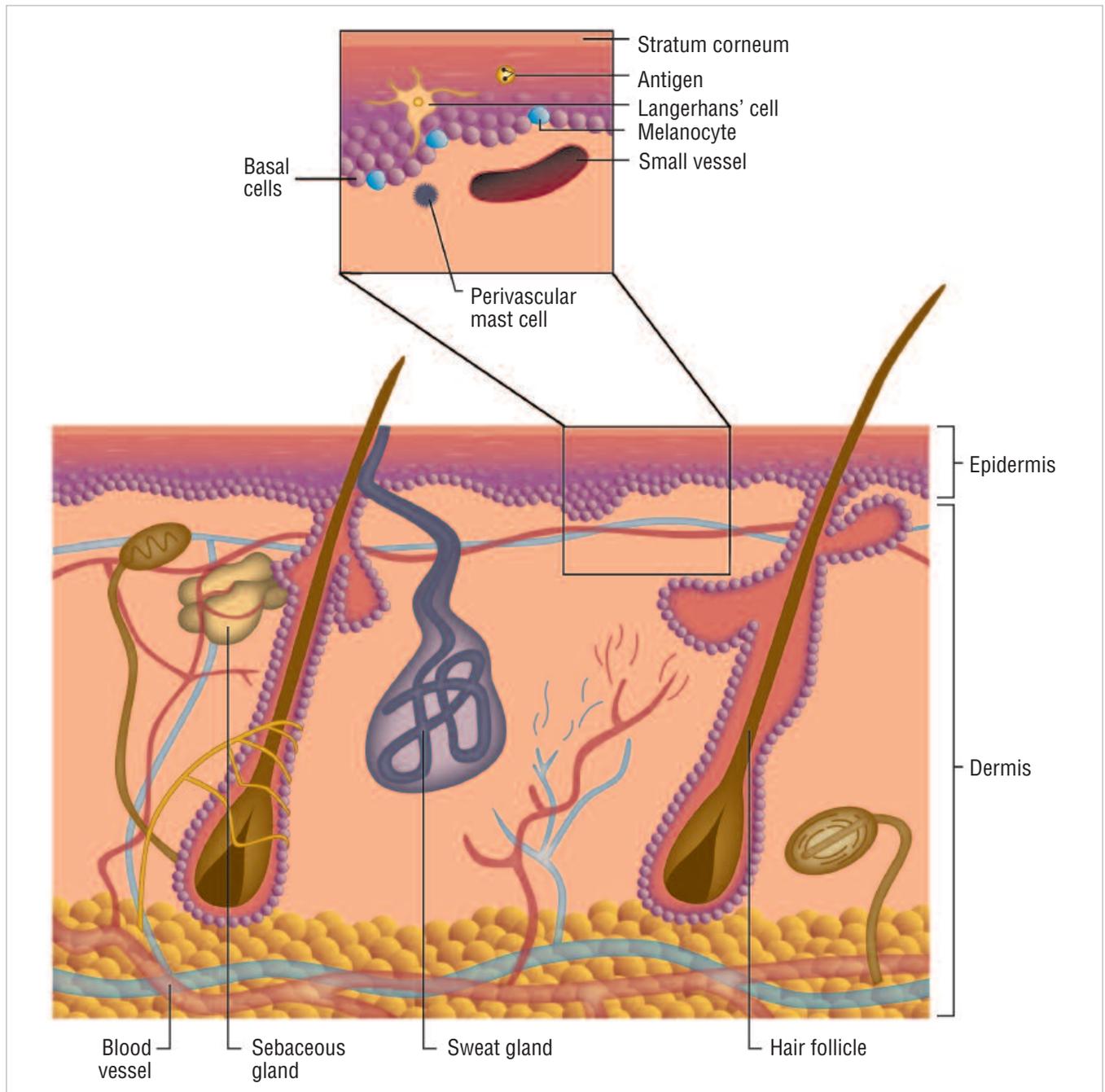


Figure 1. Diagram of the skin, which comprises the relatively thin epidermis and the thicker dermal layer. Specialized adnexal structures such as hair follicles, sweat glands, and sebaceous glands descend into the underlying dermis. The magnified portion of the epidermis demonstrates the progressive upward maturation of the basal layer of cells (i.e., stratum basale) that eventually form the stratum corneum. Langerhans' cells, melanocytes, and Merkel cells (not shown) reside within the epidermis. Mast cells and small vessels reside within the underlying dermal layer.

genitalia) and the medial surfaces of the limbs.^{3,4} The dermis also supports secondary structures, including hair follicles, sweat (apocrine) glands, sebaceous glands, blood vessels, and nerves.

Epidermis

The epidermis is a stratified squamous epithelium that originates from the ectoderm and consists of multiple layers of cells that undergo a pattern of proliferation, differen-

tiation, and keratinization. The average turnover time for a horse's epidermis to be shed and replaced is 17 days.⁵ The keratinization process starts at the germinal layer (i.e., stratum basale), where the cells undergo mitosis. As the cells migrate superficially through the stratum spinosum, granulosum, and corneum, they lose many of their cellular characteristics and act more as a mechanical barrier when they reach the stratum corneum. The stratum corneum consists of protein-rich cells containing fibrous keratin and keratohyalin encompassed by a lipid extracellular matrix (Figure 1).

Throughout the epidermis are several unique cell types, including Merkel cells, melanocytes, and Langerhans' cells. Merkel cells are located in the basal region and function as slow-adapting touch mechanoreceptors.² Melanocytes are also located in the basal region as well as in the sweat gland ducts, sebaceous glands, and outer root sheaths of hair follicles.³ Melanin pigments produced by melanocytes provide skin and hair color in horses. The color is particularly determined by the number, distribution, and degree of melanization and is controlled by genetics and melanocyte-stimulating hormone, which is secreted by the pituitary.² Langerhans' cells are commonly located in the upper spinous layer but are present in low numbers within deeper layers of the epidermis as well as dermal lymph vessels and lymph nodes.⁶ Langerhans' cells originate from bone marrow and are functionally and immunologically related to the monocyte-macrophage cell line.⁷ Langerhans' cells have been identified in horses, are proposed to function as antigen-presenting cells to lymphocytes, and act as initial receptors for cutaneous immune responses.⁶

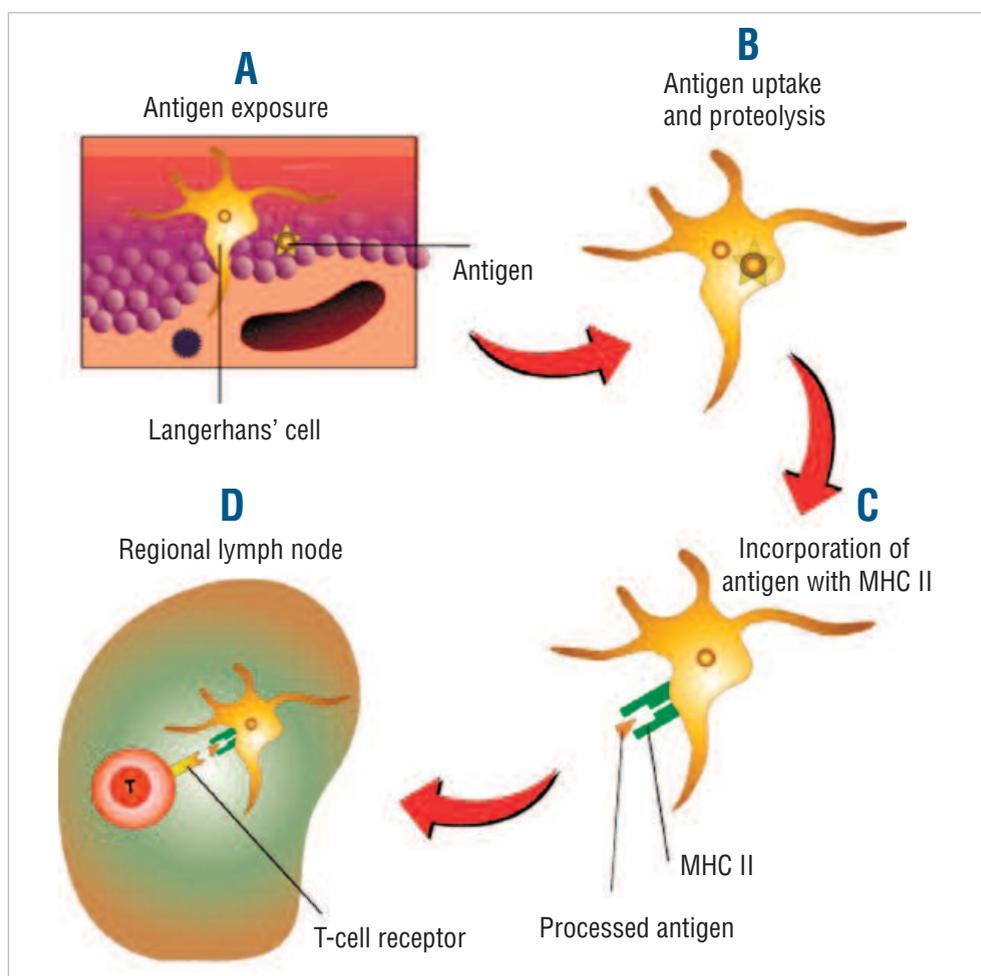


Figure 2. Diagram of antigen exposure and processing by Langerhans' cells.

A: Antigen exposure: Langerhans' cells located in the skin are exposed to antigen.

B: Antigen is incorporated into Langerhans' cells via endocytosis and degraded via proteolysis.

C: Processed antigen is presented on the surface of Langerhans' cell in association with an MHC II molecule.

D: Antigen-MHC II complex migrates to regional lymph node via afferent lymphatic and is presented to T lymphocyte.

Dermis

The dermis originates from the mesoderm and is composed of dense connective tissue, collagen, elastin, and reticular fibers that lie beneath the epidermal basement membrane, extending to the subcutis.² The dermis is divided into a papillary layer (i.e., superficial dermis) and reticular layer (i.e., deep dermis). In horses, a third layer involves the skin of the dorsal thorax, croup, dorsal surface of the back, and lateral aspect of the neck.⁸ This unique layer, located below the reticular layer, is composed of fine collagen, elastin, and reticular fibers.⁸ Smooth muscle fibers are noted within the dermis in the scrotum, teats, and penis, whereas skeletal muscle is asso-

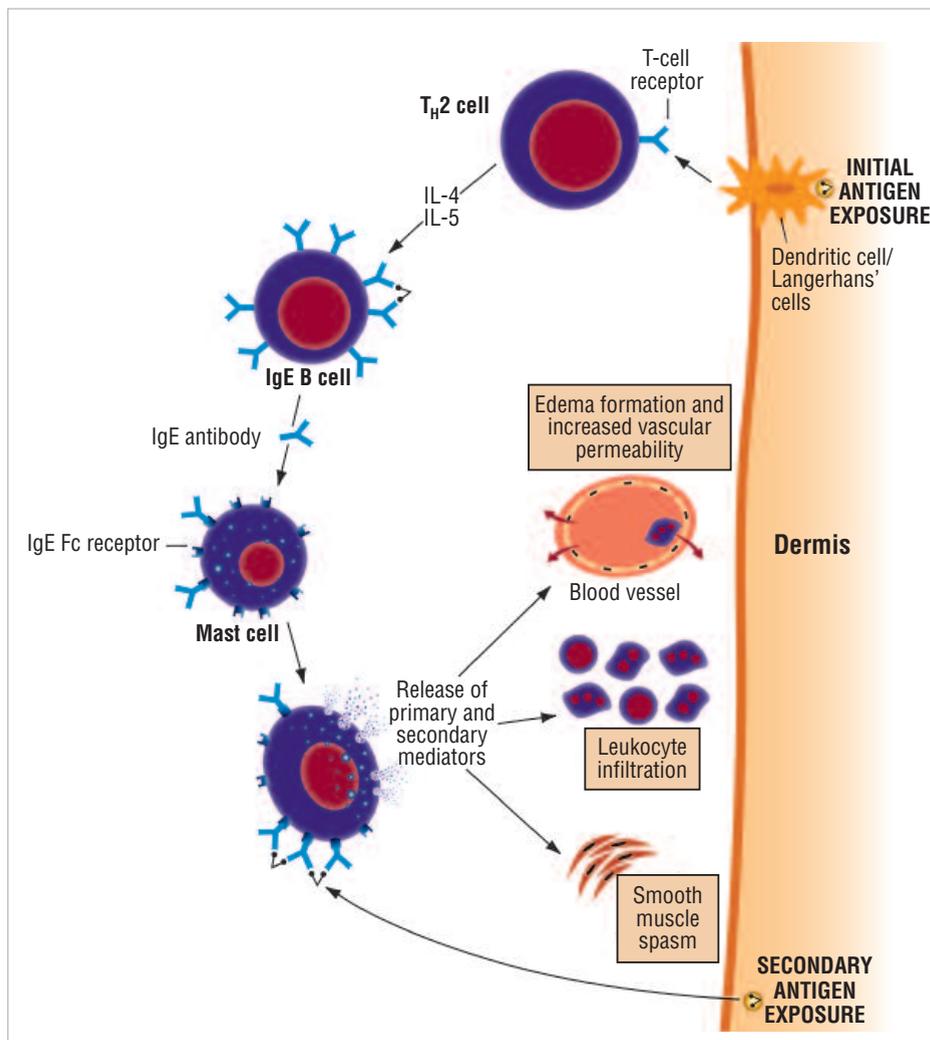


Figure 3. Development of a type I hypersensitivity reaction. Initial antigen exposure to a dendritic cell leads to processing of antigen and interaction with T helper type 2 cells (T_H2 cell). In turn, T_H2 cells produce IL-4 and 5, which stimulate B cells to produce antigen-specific IgE. IgE subsequently binds to mast cell IgE Fc receptors. During secondary exposure to specific antigen, mast cell-bound IgE is cross-linked by antigen, leading to mast cell degranulation and release of primary and secondary mediators. These mediators cause edema formation, increased vascular permeability, leukocyte infiltration, and smooth muscle spasm. A late-phase reaction may also occur 2 to 8 hours after the immediate response without additional exposure to antigen and is characterized by more intense infiltration of tissue with leukocytes.

ciated with the cutaneous trunci and large sinus hairs of the facial region. Additional structures located within the dermis include hair follicles, blood vessels, lymph vessels, nerves, sebaceous glands, and sweat glands (Figure 1).

Subcutis

The subcutis (i.e., hypodermis) is formed by a loose arrangement of collagen and elastic fibers and attaches the dermis to the deeper structures of bone and muscle.

Within these fibers are variable amounts of fat cells that provide energy, protection, support, and heat insulation to the body.^{1,2}

IMMUNOLOGIC FUNCTION

The skin acts as a peripheral sentinel for a horse's immune system and is often the first organ to encounter environmental threats at the cellular level. Of particular interest are keratinocytes, Langerhans' cells, and mast cells, all of which are commonly involved in skin diseases.

Keratinocytes

In addition to producing keratin, lipids, and intercellular substances, keratinocytes are capable of expressing a number of immunologically important surface molecules necessary for antigen presentation, such as the major histocompatibility complex (MHC) class II molecule and costimulatory molecules.⁹ Keratinocytes also produce numerous cytokines involved in cutaneous inflammatory response and a host's dermal immune system.¹⁰⁻¹² The most notable cytokine produced and stored by keratinocytes is interleukin (IL)-1.^{10,11} IL-1 is a proinflammatory cytokine released in response to cell damage and serves to initiate inflammation.¹² IL-1 also plays an important role in the immune response

by acting as a costimulatory molecule for T helper cell activation, promoting B cell maturation and clonal expansion, and enhancing natural killer cell activity and chemotaxis of neutrophils and macrophages.^{11,12}

Langerhans' Cells

Langerhans' cells are bone marrow-derived monocyte type cells and are members of a family of highly specialized antigen-presenting cells known as *dendritic cells*.⁷



Figure 4. Skin test grid before an allergen challenge.



Figure 5. Skin test reactions 30 minutes after an allergen challenge.

Langerhans' cells are the major antigen-presenting cell of the epidermis and have potent stimulatory functions for activating naive T lymphocytes.⁷ Langerhans' cells function as antigen-presenting cells via endocytosis of antigen followed by proteolysis within the cell and incorporation with MHC II molecules.^{7,13} This antigen–MHC complex migrates to the surface of the Langerhans' cell, where costimulatory molecules needed to activate the immune system are present; this complex is then presented to T cells in regional lymph nodes^{8,14} (Figure 2). Langerhans' cells are also capable of producing inflammatory cytokines such as IL-1 and 6 and play a crucial role in the development of contact hypersensitivity as noted in delayed hypersensitivity reactions.⁹

Mast Cells

Mast cells are derived from hematopoietic stem cells in the bone marrow, where they subsequently migrate through the systemic circulation until they localize in connective tissues. Mast cells are commonly found in organ systems that interface with the environment, such as the integumentary, respiratory, gastrointestinal (GI), and urogenital systems.¹⁵ Mast cells have both primary (i.e., preformed) mediators that are stored within granules and secondary mediators that are synthesized at the time of activation. The types of mediators formed within mast cells are species specific, with equine mast cells containing both histamine and serotonin.^{16,17} These mediators cause smooth muscle contraction and increased vascular permeability, leading to extravascular serum leakage and migration of neutrophils, eosinophils, basophils, and mononuclear cells to extravascular tissue^{13,17} (Figure 2).

During activation of human mast cells, synthesis of various chemokines, cytokines, leukotrienes, prostaglandins, and kinins also occurs.¹⁷ Some of these mediators, including prostaglandins, leukotrienes, and kinins, have been demonstrated in horses.¹⁷

Mast cell degranulation can be stimulated by a variety of substances, including allergens cross-linking two surface IgE molecules, complement components C3a and C5a, and eosinophil major basic protein.¹⁶ The most important cause of degranulation is cross-linking of two IgE molecules on mast cell surface receptors.^{16,18} This results in signal transduction from the surface of the mast cell to the interior, subsequent exocytosis of preformed mediators, and generation of secondary mediators¹⁶ (Figure 3). Type 1 hypersensitivity reactions, which are involved in equine insect hypersensitivity and some forms of equine recurrent urticaria, are primarily due to cross-linking of IgE molecules and subsequent degranulation of tissue mast cells.^{17,19}

DIAGNOSTIC TESTING

Various diagnostic modalities have been explored to investigate equine skin disorders. A thorough history of the duration, progression, and response to treatment(s) may provide valuable information, as does a complete physical examination. Subsequently, a specific diagnostic plan should be made to facilitate identification of the most likely disease. Although common allergic diseases such as insect hypersensitivity and recurrent urticaria are frequently diagnosed via history, clinical signs, and response to treatment, certain diagnostics (e.g., intradermal or serologic allergy testing) may be considered to

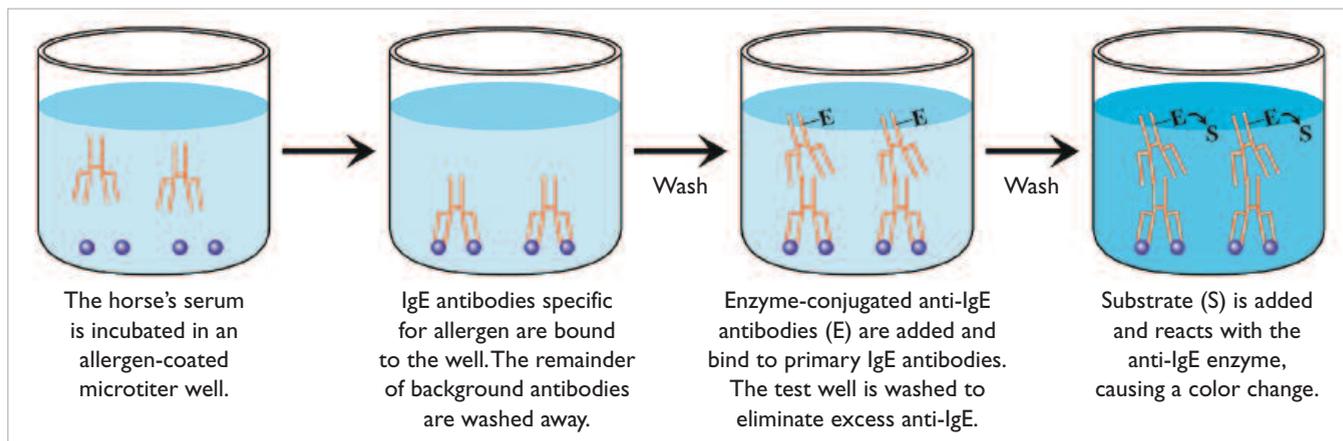


Figure 6. ELISA to determine IgE concentration. Unbound anti-IgE is washed away, and the quantity of anti-IgE bound to the solid phase is measured and converted to units.

more precisely define causative antigens. This information can then be used to develop alternative/adjunctive treatments in certain recurrent or chronic cases.

The intradermal skin test has been used to identify potential antigens involved in equine skin disorders. The intradermal skin test dates back to the early 1900s when scientists applied various substances (e.g., cat saliva, pollen grains) to mucosa or scarified skin areas, resulting in an immediate tissue reaction.²⁰ As skin testing progressed, tissue lesions were noted at the site of subcutaneous injections of tubercle bacilli in tuberculosis-infected guinea pigs. This led to the concept of the delayed hypersensitivity reaction and use of intradermal injections as a mode of delivering reactive allergens to detect allergies.²⁰ The concept of cutaneous tissue serv-

cells may represent potential causative agents in disease processes, such as food allergy, insect hypersensitivity, and recurrent airway obstruction.²¹⁻²⁶

The current intradermal skin test relies on the immunologic principle that specific injected allergens cause mast cell degranulation and histamine release in sensitized individuals. Degranulation and release of mast cell mediators lead to increased vascular permeability and a grossly visible wheal, which is interpreted as a positive reaction to that specific antigen. The wheal is typically at its largest 15 minutes after injection¹⁵ (Figures 4 and 5). A late-phase reaction to some allergens may also be observed as the immediate (i.e., 15-minute) hypersensitivity reaction begins to subside.²⁷ Various mediators released from mast cells (e.g.,

The skin has many immunologic cells that participate in local and systemic reactions and frequently manifest as skin disorders in horses.

ing as a mirror of local and systemic immune reactions was conceived through these rudimentary scratch tests. The tests work because IgE is attached to mast cells found in the skin and many other areas of the body, including the lungs and GI tract. Therefore, exogenous compounds that stimulate cutaneous mast cells may represent natural causes of allergic disease in the integumentary, respiratory, or GI system. For example, exogenous compounds that stimulate cutaneous mast

eosinophil and neutrophil chemotactic factor, IL-3 and 5) may induce a localized inflammatory reaction that begins 3 to 5 hours after intradermal injection, peaks at 6 to 12 hours, and resolves by 24 hours after the immediate response.²⁷ Evaluation of intradermal injection sites 4 and 24 hours after injection is necessary to identify antigens causing late-phase reactions.

The standard equine skin test procedure involves a negative control (i.e., 0.05 ml of 0.9% sodium chloride), a

positive control (0.05 ml of histamine at 1:100,000), and all appropriately mixed test allergens (Greer Labs, Lenoir, NC), which are injected intradermally into the horse's neck² (Figures 3 and 4). The test sites are then evaluated at 15 to 30 minutes, 4 hours, and 24 hours using a subjective five-point scale based on wheal diameter, turbidity of the wheal, and severity of erythema.² The test sites are compared with the negative (0) and positive (4+) control wheals, and all reactions that are greater than or equal to a set standard (i.e., $\geq 2+$) are considered potential causative antigens.^{2,24} Alternatively, an objective assessment can be made by measuring wheal diameter in millimeters; wheals that are greater than halfway between the diameter of the wheal produced by the negative and positive control are considered positive.² These positive reactions are then analyzed as potential sources of allergic skin disease, taking into account the horse's environment, clinical signs, and previous therapy. Avoidance strategies or hyposensitization protocols are subsequently formulated from the positive reactions identified with the intradermal skin test. The intradermal skin test has been used in horses to define specific insects involved in hypersensitivity, specific causes of recurrent urticaria and atopic (i.e., IgE-mediated) dermatitis, and potential causative antigens in recurrent airway obstruction.^{19,21-26,28,29} The skin test has not been used in diagnosing equine food allergy, but some veterinarians include some feed-related antigens in their skin test panels.

In contrast to the intradermal skin test, serum allergy tests quantify the concentration of specific serum IgE antibodies to a particular antigen. Quantification of IgE is used as a diagnostic modality in the belief that allergic individuals have a higher concentration of circulating IgE compared with nonallergic individuals. Human studies have demonstrated that serum concentrations of IgE vary widely within normal healthy individuals over time, with very low concentrations at birth and a gradual increase with age. IgE concentrations peak in the second decade of life and slowly decline thereafter.¹⁶ Human studies have also demonstrated that total serum IgE concentrations are higher in allergic individuals compared with nonallergic individuals; however, a very large overlap exists between allergic and nonallergic individuals.¹⁵ It is not known whether serum IgE concentrations follow the same general pattern in healthy horses and horses with various allergic diseases.

Most assays for determining serum IgE concentrations use immunoadsorption techniques in which a specific allergen is bound to a solid phase (i.e., plastic

Resources

Manufacturers of ELISAs for Horses

Biomedical Services

Austin, TX
Phone: 512-346-8535
Web site: www.bmslab.com

Spectrum Labs, Inc.

Tempe, AZ
Phone: 480-464-8971
Web site: www.vetallergy.com

Heska Corporation

Fort Collins, CO
Phone: 970-493-7272
Web site: www.heska.com

microtiter well). The individual's serum is then incubated with the solid phase. If the individual has antibodies to the specific antigen on the solid phase, the antibodies will bind to the solid-phase allergen. The remaining proteins and antibodies are subsequently washed away from the solid phase. The solid phase-IgE complex is then incubated with a labeled anti-IgE antibody to allow binding of the anti-IgE to any IgE bound to the solid phase. Unbound anti-IgE is washed away, and the quantity of anti-IgE bound to the solid phase is measured and converted to units (Figure 6). Both radio-labeled (i.e., radioallergosorbent test [RAST]) and enzyme-labeled (i.e., ELISA) anti-IgE have been used.

ELISAs are available for horses (see box on this page), but the value of these tests in diagnosing equine allergic disorders is not well established. Many technical variables can affect the results and reproducibility of serum tests primarily because of lack of standardization among various laboratories in preparing, mounting, and conducting tests. An extensive equine study comparing results of skin testing with those of a serum RAST and two serum ELISAs found that RAST and ELISA had poor sensitivity, specificity, and positive predictive value compared with skin testing.³⁰ Therefore, use of serologic allergy tests as a diagnostic modality remains controversial.

IMMUNOTHERAPY IN ALLERGIC DISORDERS

Results of intradermal skin and serum allergy tests have been used to develop immunotherapy protocols in select cases. Immunotherapy (i.e., hyposensitization therapy) has been used extensively in human and small animal patients to decrease dependence on steroidal

medications and alleviate or eliminate IgE-mediated allergies. Allergen immunotherapy is defined as administration of increasing quantities of allergens to patients with IgE-mediated disease.³¹ Immunotherapy may be considered when the following criteria are met: the presence of clinical signs for more than 4 to 6 months of the year, lack of response to antipruritic drugs, unacceptable drug side effects, and avoidance of antigens is impossible.^{31,32} Although the mechanism of action of immunotherapy is not clearly defined, many theories exist. It has been speculated that immunotherapy induces IgG production in secretions, serum, and tissue.^{31,32} IgG may bind and block allergens before their interaction with IgE bound to mast cells. Another theory is that immunotherapy decreases circulating IgE by stimulating suppressor T cells.³² Humans undergoing immunotherapy have demonstrated decreased IgE levels; however, this decrease does not occur in all patients who have clinical improvement. Additional theories suggest that immunotherapy may decrease the number of mast cells and/or mast cell response to antigen.³³

INTRADERMAL SKIN TESTING AND IMMUNOTHERAPY IN HORSES

Although the intradermal skin test has historically been reserved for referral clinics because of the cost, supplies, and expertise needed to conduct the test, the test may become more readily conducted in private practice settings as private hospitals continue to advance and intradermal skin test techniques become more assessable and standardized. Immunotherapy has been attempted, to a limited extent, in horses and could be a potential therapeutic modality for disorders such as insect hypersensitivity, recurrent urticaria, recurrent airway obstruction, food allergies, and other atopic diseases (e.g., recurrent pruritus).^{21–26,28,29,34–38} However, results of various equine studies involving the intradermal skin test and immunotherapy have not produced uniform results. The lack of repeatability with the intradermal skin test in diagnosing equine allergic diseases and the lack of proven efficacy of immunotherapy protocols in treating equine allergic diseases have made it difficult to accurately state their usefulness in horses.

Equine dermatitis associated with hypersensitivity to insect bites is a common and well-described skin disorder.³⁹ Intradermal skin testing and immunotherapy have demonstrated some positive results in horses with insect hypersensitivity. One study reported that immunotherapy, performed weekly, reduced clinical signs in nine of

10 horses with insect hypersensitivity during the first year of treatment.³⁴ Subsequently, the authors reported that three horses were free of clinical signs after 2 years of immunotherapy, whereas other horses had moderate (two horses) to significant (three horses) reductions in clinical signs.³⁴ Conversely, a different study identified *Culicoides* spp as the source of hypersensitivity via the intradermal skin test but subsequently found antigen-specific immunotherapy to be of minimal value because only one of six horses demonstrated improvement in clinical signs.³⁵ Additional studies have also demonstrated mixed results regarding use of the intradermal skin test and immunotherapy for insect hypersensitivity.³⁶

Equine recurrent urticaria, which is characterized by localized or generalized raised wheal formation, represents a clinical sign rather than a diagnosis and can result from reactions to drugs, feed hypersensitivities, insect bites, or inhaled allergens.^{19,26} Several studies have evaluated use of the intradermal skin test as a diagnostic method in determining potential causes of recurrent urticaria in horses.^{24–26,37} Once offending antigens are identified, avoidance strategies or immunotherapy may be used in treating recurrent urticaria. Overall, these studies have found that horses with recurrent urticaria tend to react to significantly higher percentages of total intradermal skin test antigens compared with normal horses within the first 30 minutes after injection.^{24–26} The authors of these studies caution against using positive wheal reactions to individual antigens when considering treatment because false-positive results are common; therefore, the authors suggest evaluating clinical signs, patterns of antigen reactivity, and likelihood of exposure to specific antigens when formulating immunotherapy protocols.^{24,25} One recent case report described use of the intradermal skin test followed by specific immunotherapy in a group of six related horses that demonstrated recurrent urticaria over variable lengths of time (1 month to 5 years).²⁸ Intradermal skin testing was conducted on all six horses; uniform positive reactions to antigens were not noted among the six horses, but general positive trends to similar antigens were frequently identified. These similar positive trends among the six horses included reactions to weeds, grasses, trees, molds, insects, and food-related antigens (i.e., corn and oat smut). Individual immunotherapy protocols were formulated for each horse based on intradermal skin test results, and immunotherapy was instituted using increasing doses over 70 days. Clinical signs in all horses were ameliorated with immunother-

apy without needing additional medication to control signs for 2 to 3 years.²⁸

Many investigators have also evaluated the intradermal skin test and immunotherapy as a more specific diagnostic and therapeutic for horses with recurrent airway obstruction.^{25,26,37,38} However, inconsistent results in identifying causative antigens with skin testing of affected horses coupled with variable results of immunotherapy have cast doubts on the efficacy of these methods.

Although the exact reasons for inconsistencies in skin testing results to diagnose equine allergic diseases are unknown, lack of standardization in the manufacturing of allergens, variability in antigen selection, and use of inappropriate antigen concentrations may contribute to these variable findings.⁴⁰ In addition, the most efficacious immunotherapy protocols regarding the allergens used, number and frequency of injections during the induction and maintenance phases, dose of allergen administered, and route of administration (i.e., subcutaneous, intramuscular, intradermal) are not well established in equine medicine.²

CONCLUSION

The equine integumentary system is well organized and functional, protects horses from mechanical injury, and acts as an immunologic sentinel for the body. It is clear that horses can acquire various immunologically based skin diseases. However, equine dermatology is in its infancy, and the intradermal skin test and immunotherapy have produced inconsistent results in horses. The equine studies discussed in this article present variable study designs, testing procedures, and methods of immunotherapy. Therefore, it is difficult to make definitive statements about the intradermal skin test and immunotherapy based on these studies. Nonetheless, some investigators believed that intradermal skin testing and immunotherapy were beneficial in diagnosing and treating allergic skin disease. Practitioners are often frustrated by the challenge of identifying causative agents in equine skin disorders. The value of intradermal skin testing and immunotherapy continue to be debated. As the optimal conditions for equine intradermal skin testing methods are better defined and immunotherapy protocols are specifically established, more reliable results may be achieved and stronger recommendations made.

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ARTICLE #5 CE TEST



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1. The thickest areas of equine skin are on the

- a. dorsum (i.e., head, mane, back, croup, tail).
- b. ventrum.
- c. medial surfaces of the body and limbs.
- d. genitalia.
- e. udder.

2. The dermis does not support

- a. hair follicles.
- b. sweat (apocrine) glands.
- c. sebaceous glands.
- d. subcutaneous fat.
- e. nerves.

3. The keratinization process starts at the stratum

- a. spinosum.
- b. basale.
- c. granulosum.
- d. corneum.
- e. lucidum.

4. The most notable cytokine produced and stored by keratinocytes is IL-

- a. 1.
- b. 2.
- c. 13.
- d. 6.
- e. 11.

5. Mast cell mediators

- a. are not species specific.

- b. in horses contain only histamine.
- c. cause skeletal muscle contraction.
- d. cause increased vascular permeability, leading to extravascular serum leakage and migration of neutrophils, eosinophils, basophils, and mononuclear cells to extravascular tissue.
- e. are all preformed before degranulation.

6. When intradermal skin testing was compared with serum RAST and ELISA, the latter two tests had

- a. poor sensitivity.
- b. poor specificity.
- c. poor positive predictive value.
- d. a, b, and c
- e. similar sensitivity, specificity, and predictive value.

7. Which theory regarding the mechanism of action of immunotherapy is incorrect?

- a. Immunotherapy produces allergen-specific IgG that binds and blocks allergens before their interaction with IgE bound to mast cells.
- b. Immunotherapy increases production of mast cell mediators.
- c. Immunotherapy decreases IgE levels.

- d. Immunotherapy decreases mast cell numbers or reactivity.
- e. Immunotherapy decreases circulating IgE by stimulating suppressor T cells.

8. Urticaria can result from

- a. inhalation of allergens.
- b. drug reactions.
- c. feed hypersensitivities.
- d. insect bites.
- e. all of the above

9. Which mediator has not been demonstrated in horses?

- a. prostaglandins
- b. leukotrienes
- c. kinin activity
- d. IgH
- e. histamine

10. In horses, a late-phase reaction to some allergens may peak at _____ hours.

- a. 3 to 5
- b. 6 to 12
- c. 24
- d. 36
- e. 48