Update on Equine Allergies

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KEYWORDS

• Insect bite hypersensitivity • Equine allergy testing • Food allergy • Atopy

KEY POINTS

- Horses develop several skin and respiratory disorders that have been attributed to allergy.
- These disorders include pruritic skin diseases, recurrent urticaria, allergic rhinoconjunctivitis, and reactive airway disease.
- Allergen-specific IgE has been detected in these horses, and allergen-specific immunotherapy is used to ameliorate clinical signs.
- The best understood atopic disease in horses is insect hypersensitivity, but the goal of effective treatment with allergen-specific immunotherapy remains elusive.
- The least understood is food allergy, for which there are very little hard data and no real feel for how best to diagnose it.

IMMUNOPATHOGENESIS

The term "atopy" describes an inherited predisposition to a constellation of diseases characterized by hyperreactivity to environmental allergens, including foods. These diseases in humans include allergic rhinitis and conjunctivitis, asthma, and atopic dermatitis. Production of immunoglobulin E (IgE) in response to the offending allergens is part of the disease process. The production of allergen-specific IgE is the basis of the intradermal skin testing and serum allergy testing and the findings have been used to generate allergen-specific immunotherapy with amelioration of clinical signs.[1–4] In the past, the production of IgE and its binding to the high-affinity Fc epsilon receptor on mast cells and basophils was the centerpiece of the understanding of atopy. Allergenic proteins were thought to be inhaled and somehow transported to the skin and mucous membranes, where they bound to and cross-linked cell-bound IgE. The rapid release of histamine and other preformed inflammatory mediators within minutes (type I hypersensitivity reaction) was followed by the later release of lipid mediators, such as leukotrienes and cytokines, that mediated inflammation (the late phase IgE response). Although it is known that these pathways occur, it has been learned that the

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Vet Clin Equine 29 (2013) 541–550
http://dx.doi.org/10.1016/j.cveq.2013.08.005 **vetequine.theclinics.com**
0749-0739/13/$ – see front matter

pathogenesis of atopic dermatitis in humans and dogs is much more complex, and this complexity is likely to be the case for horses too.

Most of what is known about atopic dermatitis has come from studies of the spontaneous human disease and experimental models in mice.[5–13] Many of the features described for the human disease have been verified in the canine disease as well, at least preliminarily.[14–17] The familial predisposition to atopy is associated with the potential inheritance of a large number of polymorphic genes that affect the function of the innate and acquired immune responses, as well as the structure and function of the skin barrier.[18,19] Abnormalities in the skin barrier are thought to allow for cutaneous absorption of allergenic proteins, which are taken up by dendritic cells and carried to the lymph node. Naive T lymphocytes are activated and, because the immune response is skewed toward a T-helper 2 response, IgE production is induced, and a variety of cytokines, including interleukin (IL)-4, IL-5, IL-6, IL-13, and IL-31, are released. IL-31, in particular, has garnered much interest recently because it can bind to receptors on neurons to stimulate itch. An eosinophilic inflammatory infiltrate and itch are significant features of the atopic response in the skin. Allergen-specific IgE binds not only to mast cells and basophils but also to Langerhans cells and other dendritic cells within the skin and mucous membranes and serves to capture allergen very efficiently. Subsequent exposure to the allergen results in amplification of the allergic response locally. Lack of suppression by T-regulatory cells is part of the disease process, although it is not clear whether decreased T-regulatory cell function is a cause or result of the disease process.

There is a complex interplay between the immune system and the nervous system, which promotes the sensation of itch.[10,20–23] Th2 cytokines, particularly IL-31, directly stimulate itch by binding to their receptors on nerve fibers. Other pruritogenic mediators, including histamine, proteases, substance P, opioids, neurotrophins, and other neuroactive peptides, enhance the itch. Secondary infections with Staphylococci and Malassezia yeast are frequent and further aggravate the level of itch in humans and dogs. It is thought that at least Staphylococci are involved in the impetiginization seen in horses. Much less is known about the role of Malassezia in equine atopic diseases, but the recent recognition of their role in cats suggests that mammals with atopic disease are more susceptible to these yeast infections and further work with horses is warranted. Inflammatory proteins from these microbes are absorbed more readily through the impaired skin barrier and contribute further to its breakdown. Staphylococci promote skewing toward the Th2 response, and many patients can develop IgE against bacterial and yeast proteins, resulting in bacterial and yeast hypersensitivity. Over time, as the disease becomes chronic, there is a shift toward the T-helper 1 cytokine response, with TNF-α playing a more prominent role.

It has been known for some time that the model of Th2/Th1 imbalance has been used to explain the immunologic abnormalities associated with respiratory allergies, but recent studies have shown that an epithelial barrier defect likely contributes to these diseases as well. Using biopsies from human patients with and without chronic rhinosinusitis, investigators measured trans-tissue resistance and found it to be reduced in samples from affected patients.[24] Furthermore, they showed that the components of tight junctions, occluden and zonula occudens 1, were patchy, irregular, and decreased in diseased tissue. By culturing respiratory epithelial cells in an air-liquid interface, these findings could be replicated in vitro. As well, addition of IFN-γ and IL-4 could mimic these changes when applied to epithelial cultures from normal individuals. Interestingly, a polymorphism in SPINK5 (serine protease inhibitor Kazal type 5) identified in human patients with atopic dermatitis has also been incriminated in human patients with asthma and chronic sinusitis.[19,25,26] This polymorphism is

thought to be a marker for atopy in general. SPINK5 is expressed in all epithelial tissues and is thought to inhibit function of serine proteases. Another epithelial product central to atopy is the cytokine TSLP (thymic stromal lymphopoietin).[27] This cytokine is produced by damaged epithelial cells and it is known to induce the Th2 phenotype in atopic dermatitis, allergic rhinoconjunctivitis, and asthma. Equine TSLP has been cloned, and its RNA expression has been confirmed in the bronchoalvealar lavage fluid of horses with reactive airway disease.[28,29] Recombinant equine TSLP has been expressed, and anti-equine TSLP antibodies have been generated, which will allow for the study of this cytokine in equine allergic diseases.[30] In fact, single-nucleotide polymorphisms in TSLP have been observed in horses with insect bite hypersensitivity.[31]

It makes sense to hypothesize that mechanisms similar to those in humans and dogs mediate atopic diseases in horses, and evidence is slowly accumulating. The disease most like atopic dermatitis is the pruritus and dermatitis associated with Culicoides hypersensitivity and some horses with positive intradermal skin test or serum test reactions to environmental allergens. To call the disease atopic dermatitis, it must be verified that horses make IgE in response to environmental allergens, that they have an imbalance between Th2 and Th1 cells, that they absorb allergens through the skin, and that they have an impaired skin barrier. It is very clear that horses make IgE[32–38] and that allergen-specific IgE can be detected using intradermal skin testing and/or serum testing.[39–43] Based on what is known about mammalian IgE, it can be assumed that horses, like other allergic mammals, use the same immunologic mechanisms. Although evidence is lacking with regard to pollens, molds, dusts, or danders, there is good evidence that a Th2/Th1 imbalance is involved in horses with Culicoides hypersensitivity, and that this insect bite hypersensitivity shares many features with atopic dermatitis.

Heimann and colleagues[44] used immunohistochemical staining to compare the distribution of CD4+, CD8+, and FoxP3+ T-regulatory cells between normal horses and those with insect hypersensitivity. As would be predicted, there were increased numbers of T cells in the affected horses, but ratios of FoxP3+ T cells/CD4+ were significantly lower in affected horses compared with normal horses. Cytokine expression was assessed by real-time quantitative PCR. Affected horses showed elevated mRNA levels for IL-13 in lesional and nonlesional skin, and lower mRNA levels for IL-10 in lesional skin. These data could support the hypothesis that insect hypersensitivity in horses is associated with imbalances in the ratio of T-helper 2 cytokines and those produced by regulatory T cells. However, until the expression and function of the cytokine proteins have been demonstrated, no firm conclusions can be made.

Hamza and colleagues[45–47] took a different approach by culturing equine peripheral blood mononuclear cells in the presence of mitogen, insect allergens, or irrelevant allergens. The cells were subsequently examined by flow cytometry for cytokine protein production and total cytokine was measured by ELISA. There were seasonal differences in cytokine production from horses with insect hypersensitivity. Increased production of total IL-4, increased numbers of IL-4-producing cells, and decreased production of IFN-γ were seen in the summer, when lesions were active.[45] Subsequent studies showed that reduced incidence of insect hypersensitivity was associated with down-regulation of IL-4-producing cells and increased expression of IL-10 and TGF-β.[46] Equine peripheral blood mononuclear cells from affected Icelandic horses, when stimulated with Culicoides allergen, produced lower numbers of FoxP3+ T-regulatory cells than did those from healthy horses.[47] The addition of IL-4 to the cells of healthy horses was able to reduce the number of T-regulatory cells. These data suggested that the decrease in T-regulatory cells was secondary, rather than a primary

cause of susceptibility to insect hypersensitivity. Last, as mentioned above, equine TSLP has been cloned, expressed, and antibodies against it developed, which will allow for study of the role of this cytokine in skin and respiratory allergies.[30]

Information about the genetic factors associated with insect hypersensitivities is slowly accumulating as well.[48] Differential gene expression in equine asthma is revealing potential new targets for therapy.[30,49] As these data accumulate, commonalities between horses and other animals with atopic disease should be discovered, and also interesting differences concerning the complex interplay between environment and genetics in allergic disease.

Barrier dysfunction is considered an integral part of the pathogenesis of atopic dermatitis. In fact, the skin barrier and the immune response are thought to be tightly linked.[50] Very little is known about the skin barrier of horses. An abstract recently presented at the World Congress of Veterinary Dermatology established that some of the ultrastructural changes associated with barrier defects in humans and dogs were seen in the skin of one atopic horse.[51] This finding supports continued study into the barrier function of horses, and whether barrier repair will become part of a multimodal approach to the management of atopic dermatitis in horses.

The role of food allergy in equine skin disease remains a mystery. Although food allergy is thought to occur, there is very little in peer-reviewed literature about its prevalence, its causes, or its pathogenesis. Anecdotal reports that chronic urticaria can be caused by food allergy suggests a role for IgE, but there is no hard evidence. Absolutely no information is available about the pathophysiology of food allergy in horses. Mechanisms mediating food allergy in humans can be humoral or cell-mediated. IgE-mediated disease results in the rapid onset of clinical signs after exposure to the food, whereas cellular mechanisms may have a more delayed onset. Food allergy manifestations in humans include urticaria and angioedema, but also eosinophilic esophagitis and gastroenteritis. Many of the mechanisms mediating atopic disease in skin and the respiratory tract have been demonstrated in the gut. Memory T cells specific for gut-associated allergens home preferentially to the gut on re-exposure[52] and dendritic cells have been shown to induce the Th2 response to food allergens.[53,54] Immunologic tolerance in the gut is actively mediated by regulatory T cells, which are induced by dendritic cells residing in the gut.[55] Experimental induction of food allergy requires genetic predisposition, adjuvants, and bypassing oral tolerance by exposure through other routes. These requirements are likely needed in the clinical disease as well; that is, disruption of the gut barrier by immunologic and nonimmunologic mechanisms, along with increased food allergen load, thereby setting the gut up for an allergic reaction.[56] Of interest is that newer evidence suggests that food allergy may be induced by exposure through a disrupted skin barrier and that early oral exposure actually leads to tolerance.[57] Because the gut microflora are thought to play a critical role in the establishment of oral tolerance, the use of probiotics has been studied and shown to have beneficial results in the management of food allergy.[58] The only reports of food allergy in peer-reviewed literature related to horses with recurrent urticaria are the implication that these are mediated by allergen-specific IgE.[59–61] Popular opinion suggests that pruritic skin disease might also have food allergy as a cause. The role of a Th2 helper response in equine food allergy remains to be determined.

CLINICAL DISEASE EXPRESSION, DIAGNOSIS, AND TREATMENT

Significant advances have not been seen in the diagnosis and treatment of allergic disorders in horses. Therefore a brief review of the diagnostic approach many veterinarians take with allergic horses follows.

EQUINE INSECT BITE HYPERSENSITIVITY

The most common equine allergy, and the best studied, is that to insects, in particular, Culicoides hypersensitivity.[62,63] This disease is characterized by pruritus and secondary lesions of alopecia and crusting. The distribution on the body is determined by the species of Culicoides feeding on the horse. The classic distribution is the dorsally distributed disease with lesions found on the face, mane, withers, rump, and tail ("sweet itch"). Ventrally feeding Culicoides spp cause lesions in the intermandibular space and on the ventral body wall. In some parts of the world, where there are several species of Culicoides, horses can have a combination of both. Furthermore, the species causing the disease may vary throughout the year, contributing to variation in distribution of clinical signs.[64,65]

The diagnosis of Culicoides hypersensitivity is made by history, clinical signs, response to insect control, and intradermal and/or ELISA testing (serology). An ectoparasiticidal trial for diagnosis can be difficult. These crepuscular insects feed at dawn and dusk and stabling the horses from dusk to dawn will help the problem. Fans can also be put into stalls so that feeding behavior is reduced, because these tiny flies are unable to fly and feed in brisk breezes. Ultimately, the regular application of insecticides is needed for control of this disease and permethrin seem to be the best choices.[66] Sprays are available but there are several spot-on treatments as well. Some veterinary dermatologists use the canine product Vectra 3D off-label for horses; this product contains dinotefuran, pyriproxifen (an insect growth regulator), and permethrin. Three tubes of the largest dog size are used: one applied to the mane and face, one to the back and rump, and one to the ventral abdomen. Testimonial evidence suggests that it is helpful but no published data are available.

Intradermal testing and ELISA serologic testing have been validated for this disease.[67–71]

Intradermal testing using Culiocoides extracts can be used as part of a complete panel to look at other allergic reactions. Progress has been made in identifying specific Culicoides proteins that induce immediate hypersensitivity in affected horses. In the Netherlands, 7 different proteins isolated from *Culicoides obsoletus* were used for ELISA and intradermal testing in horses.[68] This species was chosen because it is the primary species causing disease in the Netherlands. The ELISA reactions in affected horses varied from 38% to 67% depending on the individual allergen, but when all 7 proteins were used, there was a sensitivity of 92% and a specificity of 85%. These proteins were valuable in intradermal skin testing as well. When a protein from a different Culicoides spp (*C sonorensis*) was used in ELISA, IgE levels were lower. The conclusion was that for countries in which *C obsoletus* is the major parasite, these proteins could be useful for diagnosis and immunotherapy. It remains to be seen whether they would be useful for horses parasitized by other species. Similar studies using proteins from *Culicoides nubeculosis* have been performed in Switzerland.[72] Anderson and colleagues[73] in British Columbia demonstrated that immunotherapy can be effective. However, a placebo controlled study performed in Florida failed to show any benefit.[74] This clearly indicates that much more work needs to be done.

Horses can show hypersensitivity reactions to other insects as well, including blackfly, mosquito, stable fly, hornfly, and tabanids. These syndromes are less studied and most reports are anecdotal. Investigators have described allergens that cross-react between Culicoides and Simulium (black flies),[75,76] so it is possible that horses with Simulium allergies could benefit from Culicoides immunotherapy. Horses with Simulium hypersensitivity often have crusted pruritic papules where they have been bitten. Horses with Stomoxys (stable fly) hypersensitivity often have crusted pruritic

papules on their chests and limbs. Mosquito bite hypersensitivity can be associated with generalized papular urticaria or true hives. Horses with Tabanid (horse fly; deer fly) allergies can have large ulcerated masses similar in clinical appearance to mild lesions of habronemiasis. Owners often report that the lesions develop where they see horse flies or deer flies feeding. It can be quite difficult to differentiate among the various insect hypersensitivities and it is likely that some horses are allergic to multiple biting insects.

ATOPY/ATOPIC DERMATITIS

A variety of syndromes have been associated with environmental allergies in horses including pruritus, similar to that seen with insect hypersensitivities, recurrent urticaria, reactive airway disease/chronic obstructive pulmonary disease, and syndromes such as head tossing and laminitis. Some of the pruritic horses may have combined insect hypersensitivity and reactions to pollens, molds, dusts, danders, or mites.

Diagnosis can be made by intradermal testing and/or serum allergy testing. There is some support in the literature for intradermal testing but very little for serum testing.[2,4,40–43] As for dogs, intradermal testing is not standardized for horses, which accounts for the variability in our literature. Serum testing offers increased accessibility to allergy testing for horses, and if the equine testing has improved as much as the canine testing has over the last 20 years, it seems reasonable to recommend its use, as most horses will not have the opportunity to have intradermal testing.

EQUINE FOOD ALLERGY

Very little is known about food allergy in horses. All reports are anecdotal and few reports are in refereed journals.[58–61] Reported signs include recurrent urticarial, pruritic skin disease, and anal pruritus. Incriminated foods have included sweet feed, oats, corn, other grains, dry garlic, and alfalfa. Diet trials in horses are difficult. The general recommendations are to analyze what is being fed and switch to a different grain, or drop the grain entirely for the period of the diet trial. The optimal length of time for a diet trial in horses is not known. It makes sense, though, that if the clinical signs subside with a diet trial, to prove food reactivity by challenge.

SUMMARY

The understanding of allergic disease in horses is lagging behind that of dogs, but progress is being made, particularly in the area of insect hypersensitivity. It is hoped that more effective tools will be acquired for the diagnosis and treatment in the foreseeable future.

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