

Editorial

Immunoglobulin E and allergy

In this issue of *Equine Veterinary Journal*, Dupont and co-workers report on a study to evaluate the consistency of the results obtained with a commercially available immunoglobulin E (IgE)-based test for food allergy diagnosis in ponies with a provocation trial with the presumed allergens [1]. Their results suggest the IgE-based test is not a reliable screening tool for food allergy in healthy equids.

Immunological principle of IgE-mediated allergies

Immunoglobulin E-mediated allergies are characterised by the initial production of IgE against a harmless environmental substance, the allergen. The allergen-specific IgE antibodies are subsequently bound to high-affinity IgE receptors located on the cell surface of mast cells and basophils, a process called sensitisation. The immunological processes up to this point, including mast cell sensitisation, do not result in clinical signs of allergy. However, in allergic individuals, every further contact with the allergen induces rapid cross-linking of the receptor-bound IgE with subsequent release of inflammatory mediators from these cells, and initiates the immediate onset of clinical signs associated with the allergic disease. Every allergic response induces additional allergen-specific IgE antibodies resulting in the maintenance of mast cell sensitisation, an often life-long immunological allergen-specific memory and recurrent disease in affected individuals.

IgE-mediated allergies in horses

An IgE-mediated aetiology has been assumed for various equine diseases. Two diseases, summer eczema and urticaria, show all the characteristics of an IgE-mediated disease, and we feel quite confident in calling them 'allergy' [2]. Summer eczema is also described as insect bite hypersensitivity, sweet itch, Queensland itch, or *Culicoides* hypersensitivity [2] and is an allergic, seasonal dermatitis with pruritus, alopecia and excoriations at different parts of the body, mane and tail. It occurs during the summer months and is induced by various allergens from the saliva of *Culicoides* midges [3]. A number of research groups have shown that affected horses react to allergen extracts from various insects, and predominantly to those from *Culicoides* midges. The allergic reaction has been transferred to healthy horses from summer eczema affected horses via IgE followed by a *Culicoides* extract challenge. This passive transfer of the allergic reaction provided proof for the IgE-mediated aetiology of the disease [4].

Urticaria, also called hives, is characterised by typical bumpy lesions that affect large areas of the skin and are most likely to be induced by food allergens. Lesions can be round or can have multiple shapes, including linear stripes, a 'doughnut'-like or papular appearance, and other forms. Urticaria is usually not associated with pruritus but severe cases can develop a ventral oedema [5].

In various other diseases of the horse an IgE-mediated aetiology has been suspected but this remains controversial. These diseases include inflammatory airway disease (IAD), also known as recurrent airway obstruction (RAO, chronic obstructive pulmonary disease (COPD) or heaves [6]), and head-shaking [7]. Inflammatory airway disease can be induced by a hyperreactive response of the lower respiratory tract to inhaled moulds and organic dusts [6,8,9]. The disease is characterised by mucus production, interleukin-8 (IL-8) induced neutrophil accumulation in the airway, bronchial hyperreactivity and bronchospasm. Inhalation of mould and fungal antigens initiates the inflammatory airway response in susceptible horses within 6 h after exposure [10]. These characteristics clearly mark IAD as a hyperreactive response pointing to an inflammatory aetiology. Nevertheless, the involvement of IgE-mediated mechanisms in

the pathogenesis of IAD remains unclear [11]. Initial support for a role of IgE in IAD was provided by studies detecting increased *Aspergillus*-specific IgE antibodies in bronchioalveolar lavage using a polyclonal anti-IgE reagent [12]. The early finding could not be confirmed by different investigators in more recent studies using IgE serology with monoclonal IgE reagents [13,14] or intradermal testing [14–16]. Thus, although IgE-mediated mechanisms and mast cells may be involved in some IAD cases, a consistent or aetiological role of IgE in the pathogenesis of IAD is still questionable. This leads to the question: why is a possible IgE-mediated cause of a disease so difficult to confirm?

IgE responses in allergic and nonallergic horses

What distinguishes allergic horses and nonallergic? Many years ago, we thought an allergic horse develops IgE against an allergen, while the nonallergic horse does not. Today, we know it is not that simple. Although sensitisation of mast cells and basophils with allergen-specific IgE is a central event in the pathogenesis of allergy, many clinically healthy, nonallergic horses are also sensitised against various environmental substances. When tested, they show reactivity in intradermal testing or serological IgE-based assays against these allergens [5,17]. This means that nonallergic, clinically healthy horses often also develop allergen-specific IgE antibodies, at least temporarily. These antibodies can be detected in their circulation and on the surfaces of their mast cells and basophils. To the best of our current knowledge, mast cell sensitisation or serum reactivity of nonallergic horses is not a predictor nor does it point to an increased risk of developing allergy at any time [17]. In fact, most of these horses never develop allergy. Rather, allergen-specific IgE sensitisation of nonallergic horses seems to be part of their normal immune response and immune regulation to allergen exposure. In contrast to the allergic horse, which overreacts in response to the allergen and shows clinical signs, the nonallergic horse is able to determine that the allergen challenge is harmless. Consequently it down-regulates its immune response to the allergen, and remains clinically unaffected [4,18].

Allergy tests

Allergy tests are performed to confirm clinical disease and to identify allergens that are responsible for the allergic condition of the horse. Because the nonallergic horse can also be sensitised to different substances, allergy tests cannot be used on their own to diagnose allergy. The diagnosis requires clinical signs. In allergic horses insect allergens, especially those of *Culicoides* midges, are typically responsible for the induction of summer eczema, while allergens causing urticaria can be manifold but are generally absorbed through the intestine.

Today, various assays are available to detect soluble allergen-specific IgE or the sensitisation of mast cells or basophils with allergen-specific IgE [2]. Allergy testing includes intradermal testing against multiple potential allergens, *in vitro* sensitisation assays, or serological assays, most commonly enzyme-linked immunosorbent assays (ELISAs), using a variety of allergens. The goal of a serological assay is to identify IgE antibodies against the causative allergen(s) and to aid the owner with allergen avoidance or to help the veterinarian develop a management plan or desensitisation approach for the horse.

Theoretically, an IgE-mediated aetiology and the causing allergens should be easy to confirm: we just take a blood sample, submit it for allergy testing using one of several available allergy tests, and identify the allergens responsible for the allergic condition of the horse. However, in reality it is not that easy and straightforward. Unfortunately, the interpretation of allergy tests is often difficult. Numerous positive reactions

to multiple allergens are often observed, different assays show inconsistent results when the same samples are tested, and nonallergic horses frequently show positive reactions [14,19]. Consequently, all currently existing assays should be interpreted with caution and in accordance with clinical signs and history of disease.

Given the disadvantages of serological allergy tests it is reasonable to state that intradermal skin testing, despite all its disadvantages, still remains the gold standard for the identification of the inducing allergens in allergic horses. Serological assays have limitations that are difficult to overcome. These limitations have been documented in multiple comparisons of serological tests and skin testing, or those of allergic and nonallergic horses [5,13–16,19]. Serological tests are based on multiple allergens coated to the solid surface of the assay, most commonly an ELISA plate, and these allergen-coated plates are then incubated with horse serum. The available serological assays all aim for the detection of allergen-specific IgE antibodies and use a variety of IgE-specific detection reagents for this purpose. Consequently, the quality and specificity of the IgE detection reagent has a major influence on the outcome of the test. Differences in the IgE detection reagents may explain the low correlations between assay results when the same samples are compared using different serological allergy tests.

Analytical sensitivity of a serological allergy test is an even more important aspect. All horses, both allergic and nonallergic individuals, develop IgG antibodies in response to allergens [19]. Both allergic and nonallergic horses can then 'class-switch' and develop IgE antibodies to the same allergens. If the latter occurs, both allergen-specific IgG and IgE antibodies are present in the circulation of the horse in different amounts. Immunoglobulin G antibodies typically occur in the serum in the mg/ml range while IgE is present in ng/ml to low µg/ml concentrations [20,21]. Allergen-specific IgG and IgE antibodies represent only a portion of the total antibodies in serum. Nevertheless, the serum sample applied to an allergy testing plate contains approximately 1000–10,000 higher concentrations of allergen-specific IgG than IgE antibodies and these antibodies compete for binding to the allergen on the testing plate. As a result, most of the available allergen on the plate is covered with IgG, while IgE represents the minor component after serum incubation. To result in a true positive signal, the relative allergen-specific IgE concentration in a horse's serum must be high in proportion to the allergen-specific IgG and the detection reagent needs to be 100% specific for IgE. These considerations may offer an explanation for the low analytical sensitivity of serological allergy tests resulting in an accurate determination of the allergy-inducing allergens in only a few horses with rather high IgE antibody amounts.

Conclusion

In summary, there is a need for improved tools for allergy diagnosis in horses. All available assays have limitations and can result in false positive and false negative results. The use of these assays to determine allergens for desensitisation or immunotherapy in allergic horses should be re-evaluated and is questionable. New assay strategies and thoroughly characterised IgE-specific reagents are required to reliably identify IgE-mediated mechanisms in equine diseases for the development of allergy tests with improved sensitivity and specificity, and as reliable tools to determine the 'causative allergens' for desensitisation approaches in horses.

B. Wagner

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA

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