

Allergy diagnostics in horses - new diagnostic approaches the Fcε-Receptor test (Allercept™)

Introduction

Equine diseases with an allergic etiology in horses are commonly seen in veterinary practice. Clinical diseases associated with IgE mediated hypersensitivity (immediate type, type I), include

Atopy

Urticaria

pruritus and

allergic diseases of the respiratory tract (COPD)

(FADOK and GREINER, 1990; HALLIWELL, 1993; ROSENKRANTZ, 1995).

IgE mediated allergies in horses are a result of exaggerated reaction of the immune system to foreign substances. Inhalation allergens like pollen and spores of fungi or insect saliva seem to be the most common etiological agent (MARTI et al., 1997). Specific immunotherapy (SIT) and corticosteroids often provide effective treatment for these conditions (BEECH and MERRYMAN, 1986; ANDERSON et al., 1996; ANDREWS and SCHMEITZEL, 1999; HUNSINGER, 2003).

Diagnosing the allergy and identifying the allergens is crucial for a promising therapy with lasting effect. So far this often seems to be a difficult task for veterinarians.

Primarily allergies in horses are diagnosed clinically. Detailed anamnesis (breed, season, environment, feeding, possible disposition) and especially an precise observation of clinical signs and circumstances of the occurrence lead to the diagnosis.

Confirmation of the clinical diagnosis and identification of the relevant allergens is necessary for a specific immunotherapy (SIT). Theoretically this may be achieved by avoidance of allergens, by intradermal testing (IDT) or by in vitro serum allergy tests. Avoidance of allergens most often is not possible though and IDT does not provide sufficiently reliable results and is both costly and time consuming.

Intradermal testing has been regarded as “golden standard” for identification of allergens for inclusion in immunotherapy vaccines in general. Vast experience is needed though to perform and interpret an IDT, storage of allergens for injection only is economically when the test is performed frequently. Furthermore present studies show that the IDT shows unreliable results quite frequently in horses (EVANS et al., 1992; LEBIS et al., 2002; PANHUIZEN et al., 2003). A strong need exists for reliable in vitro serum allergy tests that are more accessible and available to equine practitioners (LORCH et al., 2001b).

In vitro serum allergy tests

In vitro serum allergy tests offer an alternative to the IDT for the purpose of identifying allergy causing substances by measuring circulating allergen-specific IgE antibodies in the serum. Application thus is limited to the IgE-mediated allergies (immediate type, type I-reactions).

Since accurate measurement of horse serum IgE is a valuable tool for the study and treatment of such diseases, several assays have been developed to detect allergen-specific equine IgE. However, these assays depend upon antibodies, usually polyclonal, for IgE detection (SUTER and FEY, 1983; HALLIWELL et al., 1993) or used recombinant fusion proteins for immunisation and production of antisera (MARTI et al., 1997; WATSON et al., 1997).

More recently, the Fcε-receptor test has been developed for measurement of allergen-specific IgE in horses. This serologic assay uses the recombinant alpha chain of the high-affinity mast cell receptor for IgE (Fc-Epsilon-receptor, rHuFcεR1α). The rHuFcεR1α binds specifically to IgE from a number of different species including human and horse (WASSOM and GRIEVE, 1998; BEST et al., 2000; LORCH et al. 2001a, STEDMAN et al., 2001). The Fcε-receptor allows the specific determination of antibodies that are responsible for the degranulation of mast cells. Thus this tests is closer related to the clinically observed allergy than any other IgE-test.

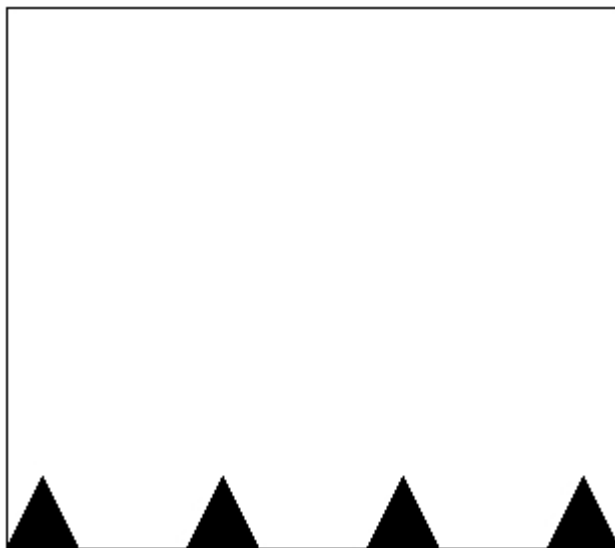
Principle of the Fcε-receptor test (Allercept™)

In vivo allergen-specific IgE is produced by B-cells after contact with allergen. Mast cells and basophiles express a protein on their surface that binds the Fc-region of the IgE with high specificity and affinity. This leads to sensitization of the cells against the specific allergen. Repeated allergen contact with mast cell bound IgE molecules is followed by cross-linking of the IgE molecules bound on the cell surface. A cascade of biochemical reactions leads to a subsequent release of inflammatory mediators, which are responsible for the clinical signs of the immediate type (type-I, IgE mediated) hypersensitivity reactions.

The Fcε-receptor test uses the high specificity and affinity of the Fcε-receptor (FcεR1α) of the mast cells and basophiles for detection of IgE in the serum. This IgE-receptor is a tetrameric complex consisting of one α, one β, and two γ subunits. The IgE molecule binds to the α subunit via the Cε3 domain in the Fc region of the immunoglobuline heavy chain. This interaction is exquisitely specific and does not bind horse IgG or IgM at all. The principle of this serum assay is given in picture 1.

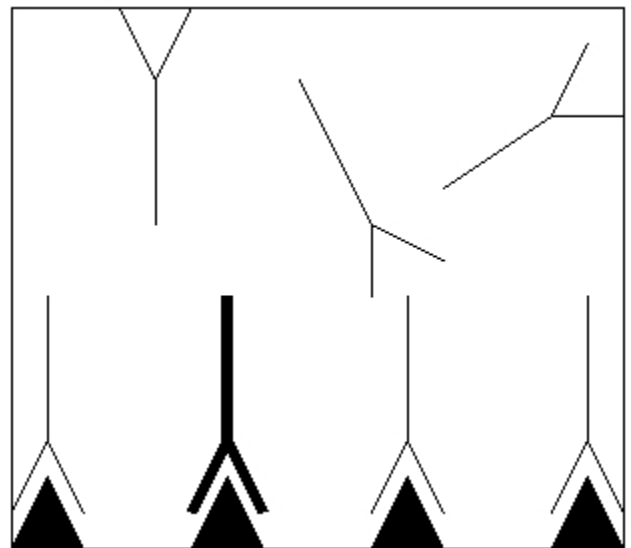
Picture 1: Principle of the Fce-Test

1.



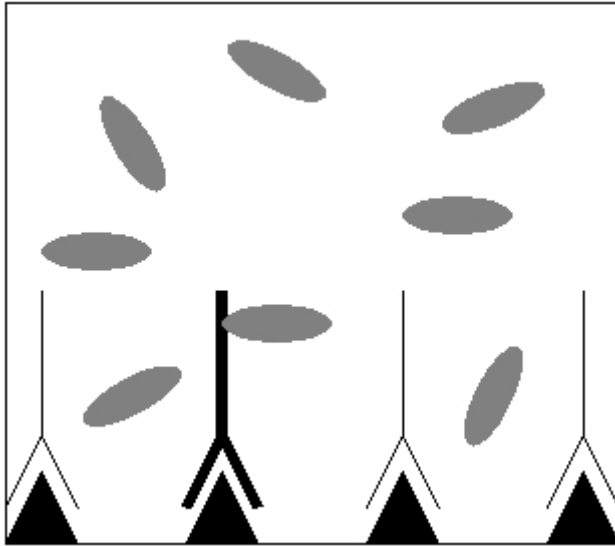
Allergens are bound on the surface

2.



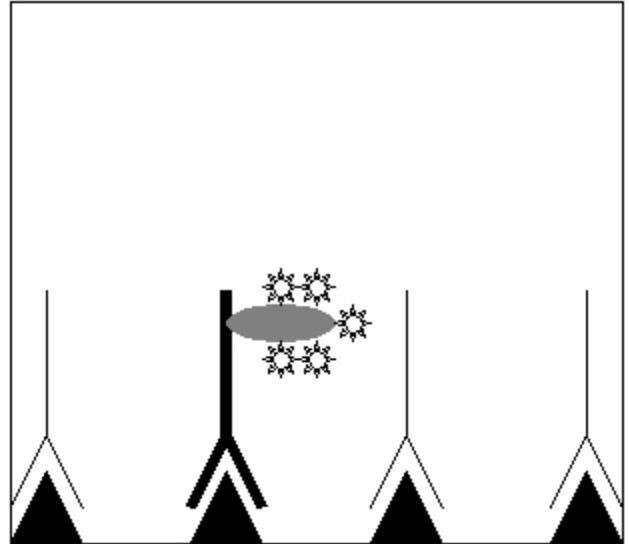
Serum of the patient is added.
Allergen-specific IgE and IgG binds the allergen

3.

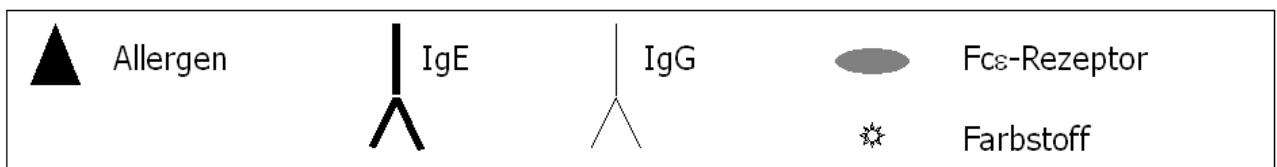


FcεR1 is added.
Receptor binds exquisitely
to IgE.

4.



Added dye marks the
bound receptor and is determined
by spectroscopy.



“False negative results”

The Fcε-receptor test detects IgE mediated allergies only – other types of allergies cannot be determined by this serum assay. allergen-specific IgE rises as a result of allergen exposure. Since free IgE antibodies (IgE not bound to mast cells) have a short half life, the test should be carried out in close relation to the occurrence of symptoms. In dogs i.e. serum allergen-specific IgE titers may decrease fast after avoidance of allergens, whereas skin hypersensitivity may persist for many months (DE WECK et al., 1997; LOESENBECK, 2000).

Allergies of the immediate type can definitely be excluded when the sample has been taken under allergen exposition and IgE was not detectable.

“False positive results”

Allergen specific IgE may be detectable in horses without clinical signs of an allergic disease due to many other factors besides IgE that have an impact on the onset of clinical symptoms (threshold value theory). Alternatively, IgE functional heterogeneity may be present in horses as it is in dogs (PENG et al., 1993; HALLIWELL et al., 1998) and humans (McDONALD, 1998). Positive results always have to be interpreted by the clinician taking into account the anamnesis. False positive results are reported for the intradermal testing too (EVANS et al., 1992; LORCH et al., 2001b; LEBIS et al., 2002).

A publication by LORCH et al. (2001b) stated that the Fcε-receptor test was superior to two other commercial equine IgE assays tested. Compared to intradermal testing as the “golden standard” the authors stated, the serum test did not reliably predicted hypersensitivity. Since in another study the authors (LORCH et al., 2001a) reported of a high percentage of non atopic horses with positive reactions in the IDT (59% of non-atopic horses were positive within 30 min following injection, 82% after 4 hours). We believe the IDT cannot be valued as golden standard as both test have to be judged according to clinical diagnosis as well as results with SIT.

Conclusion

- Prior to intradermal testing or in vitro serum assays an allergic disease always has to be diagnosed clinically
- only a limited number of allergens are tested in both intradermal tests as well as serum tests. A negative result therefore does not rule out an allergic disease as such
- in vivo or in vitro allergy tests should be used to identify relevant allergens for immunotherapy or allergen avoidance. None of them is superior to the other. None of them may identify or exclude all relevant allergens.
- Treatment success using specific immunotherapy is related to the quality of allergen selection for the treatment
- Intradermal testing is associated with several inconveniences such as sedation, clipping the hair, purchase and storage of costly allergens.
- In vitro serum allergy assays offer a good alternative intradermal testing to identify relevant allergens: they are easier accessible and feasible to equine practitioners
- The Fcε-receptor test is the only serum allergy test that identifies allergen specific antibodies that in fact have the potency to bind at the mast cells.
- Therapies based on results of the Fcε-receptor test result in successful treatment in more than 70% of the animals.