Journal of Equine Veterinary Science 32 (2012) 719-727

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Comparison of Skin Prick Tests with In Vitro Allergy Tests in the Characterization of Horses with Recurrent Airway Obstruction

Paula Tilley DVM, MSc, PhD [a], Jose Paulo Sales Luis DVM, PhD [a], Manuel Branco Ferreira DM, PhD [b]

[a] Department of Clinics, Avenida da Universidade Técnica, Lisboa, Portugal
[b] Department of Medicine II, Hospital Santa Maria, Lisboa, Portugal

ARTICLE INFO

Article history:
Received 16 October 2011
Received in revised form
26 December 2011
Accepted 28 February 2012
Available online 4 April 2012

Keywords:
IgE
Skin prick tests
Bronchoalveolar lavage fluid
ELISA
Horse

ABSTRACT

We intended to identify relevant immunoallergic factors and to compare skin prick tests (SPTs) and in vitro allergy tests in the characterization of horses with recurrent airway obstruction (RAO), so as to ascertain that SPTs perform better. Forty Lusitano/cross-Lusitano horses (30 RAO cases and 10 healthy control horses)—a very valuable autochthonous breed—were studied. Clinical history, thoracic radiography, respiratory tract endoscopy, and bronchoalveolar lavage were used for diagnosis. Serum samples of all 40 horses and undiluted bronchoalveolar lavage fluid samples of 21 RAO horses and 6 control horses were submitted for evaluation by an allergen-specific immunoglobulin E (IgE) enzyme-linked immunosorbent assay. SPTs were performed on the 40 horses. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for all diagnostic methods. Agreement between diagnostic methods was assessed by kappa statistic (K). Chi-square test with Yates correction showed SPT results from the RAO and control groups to be statistically different ($P < .05$). SPTs showed higher sensitivity, specificity, positive predictive value, and negative predictive value than both enzyme-linked immunosorbent assay tests. In human medicine, SPTs are considered to be the gold standard of allergy tests. Neither serum IgE nor bronchoalveolar lavage fluid IgE reliably detected allergen hypersensitivity, compared with SPT. SPTs performed significantly better overall than both in vitro tests. Low sensitivity of the in vitro assays indicates the need for continued study to elucidate a more sensitive specific IgE test.

1. Introduction

Respiratory disease, and in particular recurrent airway obstruction (RAO), continues to be a major problem for horse industries worldwide [1]. Medical history and physical examination are not totally reliable to exclude other diagnoses or characterize airway impairment. Recent data suggest that allergy may play an important role in RAO in horses, similar to humans, and more objective assessments are still necessary for a more accurate diagnosis.

Developed countries are suffering from an epidemic rise in immunologic disorders such as allergy-related diseases and certain autoimmune diseases. The "hygiene hypothesis" suggests that a lack of exposure to bacterial antigens, which would otherwise lead to a more T helper 1–dominated response with increased total immunoglobulin E (IgE), combined with low levels of parasite-specific IgE will reduce the threshold at which IgE responses to environmental antigens can sensitize mast cells [2-4]. In humans, an inverse relationship between susceptibility to asthma and resistance to parasites was demonstrated, and in horses, RAO was associated with resistance against strongyloid parasites in a high-prevalence family [5].

Horses naturally develop type I hypersensitivity diseases, and today, skin hypersensitivity has been confirmed to have

Corresponding author at: Paula Tilley, DVM, MSc, PhD, Equine Unit, Department of Clinics, Avenida da Universidade Técnica, Polo Universitário da Ajuda, 1300-477 Lisboa, Portugal.
E-mail address: paula_tilley@sapo.pt (P. Tilley).

0737-0806/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jevs.2012.02.018

an IgE-mediated allergic etiology [3,6-8]. Although there is still some controversy, a number of studies suggest that IgE-mediated immediate-type allergic reactions are associated with RAO. T helper 2–type cytokine-mediated chronic airway pathology (increased expression of interleukin 4 mRNA and decreased expression of interleukin 2 mRNA), including goblet cell metaplasia in small bronchioles with massive mucus overproduction, is known to be involved. This is consistent with the hypothesis that RAO is an allergic condition similar to human asthma [9-13].

Immunohistochemistry studies using monoclonal anti-IgE showed the presence of IgE-positive cells in the lung tissue of RAO horses [14]. Also, RAO-affected horses, like asthmatic humans, had significantly more chymase-positive mast cells in the walls of bronchi and bronchioles than control horses. The significant relationship between high numbers of chymase-positive mast cell in the bronchial wall and lung fibrosis suggests that these may be involved in tissue remodelling. Furthermore, high numbers of chymase-positive mast cell were also associated with increased infiltration with lymphocytes and neutrophils [14,15].

Horohov et al. [16] showed that RAO-affected horses exhibit varying degrees of T helper 1– and T helper 2–type cytokine production with time (for instance, whether they are environmentally exposed to aeroallergens), so an increase or decrease of IgE-positive cells in bronchoalveolar lavage fluid (BALF) could be transient.

Immunoglobulin G (IgG)(T)—a fraction of equine IgG composed of IgG3 and IgG5—binding to skin mast cells induces degranulation and contributes directly to clinical allergy, mediating immediate skin reactions in horses. IgG4 is the isotype mainly correlating with IgE levels in humans [8,17-22], where a specific function of IgG4 in serum might be to control antigen recognition by IgE and, consequently, to regulate anaphylactic reactions and IgE-mediated immunity [23].

Physiological concentration of total IgE is approximately 1,000-fold higher in horse than in human serum [8]. IgE is also found on the surface of equine peripheral blood cells, including basophils (32%), subpopulations of B cells and monocytes (66%), and eosinophils (2%), and on mast cells in various tissues such as the skin and the submucosa of the airways and intestine [6-8,22,24,25]. The synthesis of IgE starts at 6 months of age, by which time it is established whether foals will have relatively high or low IgE as adults [3,6]. Genetic factors (major histocompatibility complex class I antigens or genes linked to the major histocompatibility complex influence the production of allergen-specific IgE) and the environment influence allergen-specific IgE production [6].

Eder et al. [6] found highly significant ($P < .001$) stud farm effects on serum IgE levels against five tested allergens.

Steinbach et al. [20] suggested and Wagner et al. [22] provided the first direct evidence that IgE binds to high-affinity IgE receptors (FcεRI) on basophils and on skin mast cells and mediates classical type I allergy in horses, thereby playing a key role in allergic inflammatory responses induced by mast cell degranulation; published data suggest that IgG(T) can contribute directly to clinical allergy, mediating immediate skin reactions in horses. IgG(T)—a fraction of equine IgG composed of IgG3 and IgG5—can bind to high-affinity Fcγ receptors on equine skin mast cells, activating degranulation. IgG binding to canine mast cells through Fcγ receptors has also been reported [8,22].

In fact, horses with detectable IgE titers against recombinant (r)-*Aspergillus fumigatus* had significantly higher IgG titers (particularly IgG1) against this r-allergen than horses with undetectable IgE titers. Allergen-specific IgE (r-*A fumigatus*) and, more strongly, IgG1 are associated with the RAO phenotype [7,18].

Histamine release assays with mast cells from BALF also suggest an involvement of IgE-mediated reactions leading to increased mast cell degranulation in RAO [26].

The use of skin prick tests (SPTs) and in vitro allergy tests in the identification of horses with RAO is presented in this article.

SPT should be a nontraumatic (blood-free) procedure used to confirm the diagnosis of immediate hypersensitivity reaction so as to select eviction measures and/or specific immunotherapy. After allergen inoculation, allergen-specific IgE binds to the high-affinity receptor FcεRI on mast cells and a complex signal transduction cascade is activated. This eventually culminates in mast cell degranulation, with the release of a variety of preformed inflammatory mediators and development of a wheal-and-flare reaction [27].

Allergen-specific IgE enzyme-linked immunosorbent assay (ELISA) helps quantify allergen-specific IgE levels and can be used with serum or BALF samples. The Fcε receptor test uses the recombinant α chain of the high-affinity mast cell receptor for IgE (Fcε receptor, rHuFcεR1α) [28].

The authors intended to identify relevant immunoallergic factors and to compare SPTs and in vitro allergy tests in the characterization of horses with RAO, so as to ascertain that SPTs perform better.

2. Material and Methods

2.1. Horses

All 40 horses were privately owned Lusitanos or cross-Lusitanos, a very valuable autochthonous breed. Age was not significantly different between the two groups ($P = .25$; t test), with a normal distribution (Shapiro–Wilk test). Average age and standard deviation was 14.68 ± 3.98 (years) in the RAO group and 16.50 ± 4.63 (years) in the control group (Fig. 1).

Thirty horses with RAO and 10 healthy control horses were selected and studied according to the phenotypic description of RAO [29], taking into account medical history and physical examination, additionally supported by respiratory endoscopy, thoracic radiography, and BALF cytology and total protein concentration [30].

Taking into account the reversible airway obstruction induced by exposure to organic dust, which is characteristic of RAO [29], all 30 affected horses included in this study had been environmentally exposed—by being exposed to a particulate-rich environment that triggered inflammation and airway obstruction—before entering the Veterinary Teaching Hospital. This is to say that they were always examined in exacerbation.