

***In vitro* bioassay to detect reaginic antibodies from the serum of horses affected with Recurrent Airway Obstruction**

Gabriel Morán · Rafael Burgos · Oscar Araya · Hugo Folch

Accepted: 26 November 2009

© Springer Science+Business Media B.V. 2009

Abstract In horses, Recurrent Airway Obstruction (RAO) is an allergic disease that involves IgE mediated Type I Hypersensitivity responses. The development of this type of allergy involves a series of events that begins with reaginic antibodies, mainly IgE and some IgG subclasses. These reaginic antibodies bind with high affinity, via the Fc portion, to FcεRI receptors on the membrane of mast cells and basophils. Once bound, environmental allergens cross-link the antibodies, which results in mast cell degranulation leading to the production of histamine and other chemical mediators that act together to induce airway inflammation. RAO-affected horses present with coughing, respiratory distress, airway obstruction and poor performance. The aspect of the RAO has been extensively studied, yet the precise sequence of events is still not well understood. Therefore, this study proposes a bioassay for reaginic antibody detection from horse serum of RAO-affected individuals, in order to determine the etiology of disease, which mediate immediate type reactions. The technique involves measuring *in vitro* calcium mobilization in RBL-2H3 cells following incubation with horse serum from affected or unaffected horses and one of the RAO antigens (*Aspergillus fumigatus*). The results presented here demonstrate that 30% of RAO-affected horses react positively in this *in vitro* bioassay, whereas unaffected horses do not. This bioassay may facilitate further research on RAO and other allergic diseases in horses.

Keywords RAO · Reaginic antibodies · RBL-2H3 cells · *Aspergillus fumigatus* · Horses

G. Morán (✉) · R. Burgos

Department of Pharmacology, Faculty of Veterinary Science, Universidad Austral de Chile, Valdivia, Chile

e-mail: g Moran@uach.cl

O. Araya

Department of Clinical Veterinary Science, Faculty of Veterinary Science, Universidad Austral de Chile, Valdivia, Chile

H. Folch

Department of Immunology, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile

Abbreviations

BALF	bronchoalveolar lavage fluid
FCS	fetal calves serum
HBSS	hank's balanced salt solution
PBS	phosphate buffer solution
RAO	recurrent airway obstruction

Introduction

It is known that reagenic antibodies have played an important role in the induction of type I immediate hypersensitivity reactions ever since Ishizaka demonstrated its causal role in arming mast cells to respond to antigenic stimulation (Ishizaka and Ishizaka 1966). These antibodies, mainly IgE and some IgG subclasses, play an important role in defense against parasites in all animal species, but they also participate in the pathogenic events leading to many allergic diseases (Wagner et al. 2003). In horses, IgE mediated type I hypersensitivity is a common pathogenic element present in several immune-mediated diseases such as summer eczema, urticaria, and head shaking, as well as Recurrent Airway Obstruction (RAO). In the development of RAO, the activity of reagenic antibodies is dependent upon genetic susceptibility as well as the type, quantity, and time of environmental allergen exposure (Halliwell et al. 1993; Schmallenbach et al. 1998; Eder et al. 2000). Antigens most commonly implicated are *Aspergillus fumigatus*, *Faenia rectivirgula* (McPherson et al. 1979; Derksen et al. 1988), *Alternaria* spp (Hare et al. 1999) and *Mucor* spp (Dirschel et al. 1993). Aside from the typical hypersensitivity type I that triggers the RAO condition, once the disease is established then typical components of type III hypersensitivity appear, such as the accumulation of polymorphonuclear leukocytes, probably due to antigen-antibody complex formation in the respiratory tract (Franchini et al. 2000; Lavoie et al. 2001). Similar to other allergic diseases, in RAO-affected horses, reagenic antibodies bind through their Fc fragment to high affinity receptors (Fc ϵ RI) present in the membrane of mast cells and basophils. The sensitizing antibodies bound to the mast cell membrane are subsequently cross-linked by allergen exposure, which results in mast cell degranulation with the production of histamine, serotonin, leukotrienes, tromboxane, and 15 HETE that in turn induces airway inflammation (van der Haegen et al. 2005). Consequently bronchospasm, mucus secretion, bronchiolar wall thickening, and obstructions in the respiratory tract are common (Derksen et al. 1985; Gerber et al. 2004). RAO-affected horses present with coughing, respiratory distress, reversible airway obstruction and reduced performance (Derksen et al. 1985).

Previously, several studies have investigated the role of reagenic antibodies in the pathogenesis of RAO and other allergic disease (Matthews et al. 1983; Halliwell et al. 1993; Schmallenbach et al. 1998; Eder et al. 2000). However, these investigations have been hampered by problems with the use of antibodies for detection of equine IgE (Tahon et al. 2009). Actually, few studies have been performed with monoclonal antibodies (Wagner et al. 2003) or with the α chain of the IgE receptor (Lorch et al. 2001a) to allow specific detection of equine IgE. Additionally, direct testing of the horse's skin has not proven useful for diagnostic purposes since most horses give a positive skin reaction to common barn allergens, even though horses with RAO tend to have a stronger positive skin reaction (Eyre 1972; Schatzmann and Gerber 1972; Evans et al. 1992; Mcgorum et al. 1993; Lorch et al. 2001b). Furthermore, another study suggests that a higher concentration of *A. fumigatus* mixture in the skin test may be needed to detect horses sensitive to this antigen group (Wong et al. 2005).

The aspect of the RAO has been extensively studied, yet the precise sequence of events is still not well understood. The purpose of the present study is to investigate a serum-based *in vitro* bioassay, which activates basophilic leukemia cells (RBL-2H3), for the detection of horse specific reaginic antibodies (in this case to *A. fumigatus*). This activation is noted by monitoring intracellular Ca^{2+} mobilization as previously described (Kim et al. 1997; Ali et al. 2001; Ching et al. 2001).

Material and methods

Horses

Thirty horses with clinical diagnoses of RAO (RAO herd) and eight negative control, normal horses (ranging from 4 to 20 years old and 420 to 450 Kg) of Chilote and Chilean criollo breeds were used in this work. This study was conducted avoiding animal distress and with the approval of the Bioethical Committee of the University.

RAO was diagnosed after careful physical examination (including rectal temperature, heart rate, respiratory rate, evaluation of hydration, gut motility, and auscultation of heart and lung), hematology analysis, endoscopic inspection and bronchoalveolar lavage fluid (BALF) examination in order to establish the clinical score for RAO as previously described (Robinson et al. 2000). It was also important to rule out other lung pathologies or parasitism. RAO-affected horses developed typical disease symptoms such as cough, increased respiratory effort, tracheal mucus accumulation and increased neutrophils in the BALF. None of the control horses had a history of respiratory disease for the preceding five months, and the clinical examination, the endoscopic examination, and the BALF cytology showed no abnormal respiratory signs.

Blood samples

Prior to performing the bioassay, blood samples were collected by jugular venicapture into sterile tubes. For preparation of serum, the tubes were kept 30 min at 37°C and centrifuged 7 min at 650×g. The serum was kept frozen at −20°C until used.

Antigenic preparations of *A. fumigatus*

The culture of *A. fumigatus* was carried out in two steps. First, the fungus was cultured in peptone-agar for 7 days at 37°C and then in malt broth for an additional 7 days. The resulting fungus was used for soluble protein extraction. To extract the proteins from *A. fumigatus* cultures, the fungus was blended and subsequently sonicated (Ultrasonic Homogenizer, series 4710 Cole Parmer, USA), the resulting suspension was centrifuged at 12,500×g for 10 min, and the supernatant containing the soluble proteins was kept at −20°C until used.

RBL-2H3 cell line

RBL-2H3 cells were grown in RPMI-1640 medium (Sigma, USA) supplemented with 10% fetal calf serum plus (FSC) antibiotics. The cells were maintained at 37°C in a 5% CO₂ humidified chamber. RBL-2H3 cells were used just before they reached confluency in culture.

Ca²⁺ mobilization in RBL-2H3 cells

RBL-2H3 cells were adjusted to a concentration of 10^6 cells/ml in RPMI-1640 medium containing 10% FSC plus antibiotics and incubated for 2 hours at 37°C in the presence of 200 µl of horse serum obtained from RAO-positive or normal horses. In addition, cells incubated without horse serum were used as a control. Next the cells were washed twice and resuspended in 1 ml of Hank's balanced salt solution (HBSS) and later incubated in the presence of 4 µl of fura-2 (2 µM) (Molecular Probes, the USA) plus 100 µM sulfinpirazone (Sigma Chemical Co, the USA) at 37°C for 30 min. Finally, the cells were washed twice and resuspended in HBSS at a concentration of 10^6 cells/ml with 0.1 µM calcium chloride (Sigma Chemical Co, the USA) and placed in spectrofluorimeter quartz cuvettes, and the fluorescence was recorded. The basal fluorescence signal of the cell suspension was measured before and after being pulsed with anti-total horse immunoglobulins (antibodies prepared in rabbit) or 77 µg of soluble protein obtained from *A. fumigatus* added in a volume of 100 µl. The control level of total Ca²⁺ mobilization was induced with 30 µl of 0.5 µM digitonine (Sigma Chemical Co, the USA), followed after a few seconds with 2 µl of 1 µM EGTA (Sigma Chemical Co, the USA) to chelate the free Ca²⁺. Fura-2 fluorescence was monitored with a fluorescence spectrofluorimeter (LS55, Luminescence Spectrometer, PERKIN ELMER) using an excitation light of wavelength 340 and 380 nm and reading the emission at 505 nm. During the measurement process, the cell suspension was maintained at 37°C.

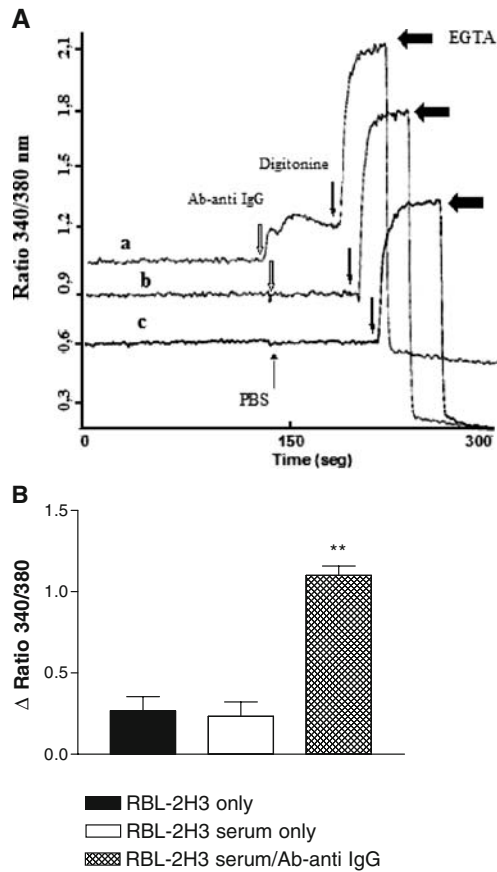
Data analysis

The absolute values of the Δ Ca²⁺ free concentration for graph generation and statistical analysis were performed using Prism software (version 5.0; Graphpad). Differences between groups were determined by using Tukey's Multiple comparison test.

Results

When RBL-2H3 cells were incubated with fresh horse serum followed by anti total horse immunoglobulins, a rapid Ca²⁺ mobilization was noted. The anti-horse IgG antibodies induced a clear increase in fluorescence emission in the sensitized cells, demonstrating Ca²⁺ mobilization. Alternatively, the signal did not change when the cell population was not sensitized with horse serum and instead received only the anti horse immunoglobulin. The same was also true for the control where fura loaded cells, sensitized with horse serum, received only phosphate buffer solution (PBS). The Ca²⁺ mobilization was corroborated by the 340/380 ratio emission (Fig. 1b), the ratio of RBL-2H3 with horse serum plus antibody total IgG, being statistically significant ($P < 0.001$) in comparison to other groups. To determine whether Ca²⁺ mobilization can be used as an *in vitro* bioassay to determine the antigen responsible for inducing RAO in a given individual (knowing that *A. fumigatus* is a widely distributed allergen in the region), sera from 30 horses with an RAO diagnosis and 8 sera samples obtained from clinically normal controls were used to sensitize RBL-2H3 cells as described above. The cells were labeled with fura and subjected to the antigen under study. The results showed that out of the 30 RAO-positive animals, 9 were positive for *A. fumigatus* (30%) while all the healthy control animals were negative. Fig. 2a shows a representative result for an animal positive with *A. fumigatus* and five animals negative for this antigen. For the 340/380 emission ratio (Fig. 1b), the ratio of RAO-affected horses

Fig. 1 **A** Ca^{2+} mobilization in RBL-2H3 sensitized with fresh horse serum challenged with rabbit IgG anti-total horse immunoglobulins (a), compared with non-sensitized RBL-2H3 cells exposed to anti-total horse immunoglobulins (b) or sensitized RBL-2H3 cells exposed to PBS (c). **B** Absolute value of the ΔCa^{2+} free concentration (** $P < 0.01$)

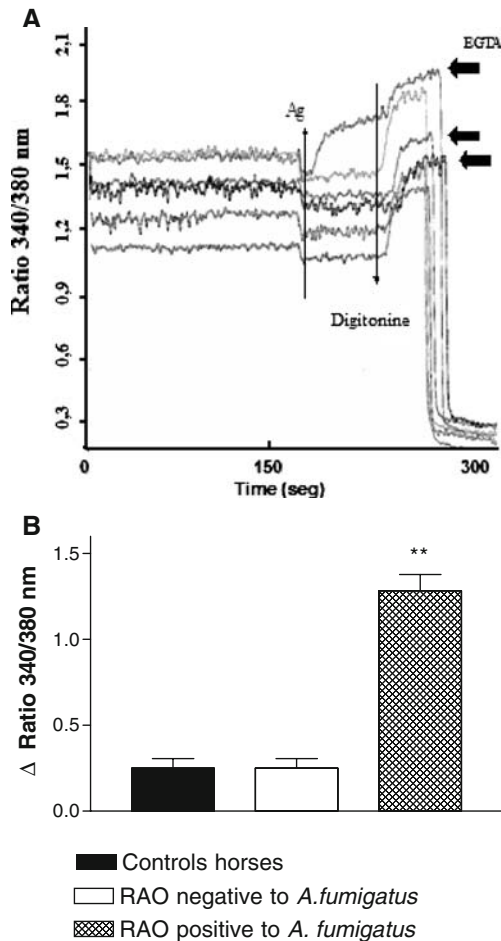


sensitized to *A. fumigatus* was statistically significant ($P < 0.001$) in comparison to RAO-affected horses not sensitized to *A. fumigatus* and other control groups.

Discussion

The aim of our study was to investigate an *in vitro* bioassay for the specific detection of reagenic antibodies in RAO-affected horses. It is generally accepted that mast cells play a central role in the initiation of the symptoms associated with RAO (Van der Haegen et al. 2005) and other allergic disorders in horses (Morgan et al. 2007). The reactions are a direct consequence of the release of pro-inflammatory mediators following antigen-dependent aggregation of occupied high-affinity IgE receptors (Tkaczyk and Gilfillan 2001). In this work, we used the generic name of reagenic antibodies to encompass all immunoglobulin molecules that can sensitize rat mast cells. Although IgE is greatly responsible for immediate hypersensitivity, other subclasses of IgG might mediate mast-cell degranulation in humans and horses; it has been recently described that IgGT can mediate histamine release from equine mast cells (Hellberg et al. 2006). In this case, we do not know which immunoglobulin isotype is mediating the phenomenon reported here, but the fact that the sensitizing capacity of the horse serum disappeared after being heated to 56°C for 30 min is

Fig. 2 **A** Ca^{2+} mobilization obtained in RBL-2H3 cells sensitized with a serum from RAO-affected horse positive and negative to *A. fumigatus*; or with serum control animals. **B** Absolute value of the ΔCa^{2+} free concentration (** $P < 0.01$)



consistent with the thermosensitivity of the IgE molecule (Prouvost-Danon et al. 1977), a characteristic that is generally absent in the IgG subclasses. Based on these facts, we assume that the IgE immunoglobulin plays an important role in this *in vitro* phenomenon.

Rat basophilic leukemia RBL-2H3 cells originate from mucosal mast cells and are a major model for the study of IgE-mediated degranulation and production of inflammatory factors present in immediate hypersensitivity (Metzger 1992; Adamczewski and Kinet 1994; Scharenberg and Kinet 1995; Frigeri and Apgar 1999). These cells have an important distinction from fresh isolated mast cells; they adhere strongly to the plastic or glass surface where they are being cultured and show a low number of small granules, making the degranulation process difficult to see under a light microscope; however, as demonstrated here, these cells become activated once the Fc receptors are occupied and crosslinked. Ca^{2+} mobilization occurs as a consequence of this cell activation, as previously reported (Kim et al. 1997; Ali et al. 2001; Ching et al. 2001), and this cellular event serves as the basis of the bioassay that we propose for the detection of specific reaginic antibodies against the allergen causing RAO in a given horse. Using this method, we demonstrated that, out of 30 cases of RAO-positive horses arriving at our hospital, 30% were confirmed to be caused by *A.*

fumigatus. Studies suggest that RAO-affected horses have higher IgE levels against *A. fumigatus* and *Faenia rectivirgula* in BALF extracts compared to healthy controls (Halliwell et al. 1993; Schmallenbach et al. 1998). Although no differences were found in serum IgE levels against mold allergen extracts between RAO-affected and control horses (Halliwell et al. 1993; Schmallenbach et al. 1998; Eder et al. 2000), Eder et al. (2000) showed that RAO-affected horses have higher serum IgE and IgG levels against pure recombinant *A. fumigatus* allergens and that the stud-specific environment exerts a highly significant effect on allergen-specific serum IgE levels (Eder et al. 2001). In similar studies, Künzle et al. (2007) showed that RAO-affected horses have higher serum IgE levels (57%) against recombinant *Aspergillus* (rAsp f 8). The results in this study show the importance of this antigen in the etiology of RAO in our region, which is corroborated by a recent report from our group showing that healthy and RAO-affected animals have specific circulating antibodies against the crude mold extract *A. fumigatus* as determined by ELISA (Moran et al. 2009). Therefore, we speculate that, if all the animals have been exposed to this wide-spread antigen, other intrinsic factors, like MHC haplotype and cytokine production, might favor the TH-2 response (Marti and Harwood 2002). Additionally, external factors, like parasitic diseases known to drive the immune response to the humoral side, or stabling conditions, which may determine the route, amount and timing of antigen immunization in each case, can also affect the outcome (Jackson et al. 2004; Horohov et al. 2005).

In summary, the present study suggests that reaginic antibodies, which mediate immediate type reactions, are the immunological mechanism leading to RAO. We conclude that the *in vitro* bioassay proposed here may facilitate further research of RAO and other allergic diseases in horses. However, studies are needed to investigate the clinical significance of reaginic antibodies in RAO affected horses.

Acknowledgements This work was supported by DID- UACH-S-2009-22 of Universidad Austral de Chile

References

- Adamczewski, M. and Kinet, J.P. 1994. The high affinity receptor for immunoglobulin E. *Chem. Immunol.* **59**, 173–190.
- Ali, H., Maeyama, K., Sagi-Eisenberg, R. and Beaven, M.A. 2001. Antigen and thapsigargin promote influx of Ca^{2+} in rat basophilic RBL-2H3 cells by ostensibly similar mechanisms that allow filling of inositol 1,4,5-triphosphate-sensitive and mitochondrial Ca^{2+} stores. *Biochem J* **304**, 431–440.
- Ching, T.T., Hsu, A.L., Johnson, A.J. and Chen, C.S. 2001. Phosphoinositide 3-kinase facilitates antigen-stimulated $\text{Ca}(2+)$ influx in RBL-2H3 mast cells via a phosphatidylinositol 3,4,5-trisphosphate-sensitive $\text{Ca}(2+)$ entry mechanism. *J Biol Chem* **276**, 14814–14820.
- Derksen, F.J., Robinson, N.E., Armstrong, P.J. and Slocombe, R.F. 1985 Airway reactivity in ponies with recurrent airway obstruction (“heaves”). *J Appl Physiol* **58**, 598–604.
- Derksen, F.J., Robinson, N.E., Scott, J.S. and Stick, J.A. 1988. Aerosolized Micropolyspora faeni antigen as a cause of pulmonary dysfunction in ponies with recurrent airway obstruction (heaves). *Am J Vet Res* **49**, 933–938.
- Dirschel, P., Grabner, A. and Buschmann, H. 1993. Responsiveness of basophil granulocytes of horses suffering from chronic obstructive pulmonary disease to various allergens. *Vet Immunol Immunopathol* **38**, 217–227.
- Eder, C., Cramer, R., Mayer, C., Eicher, R., Straub, R., Gerber, H., Lazary, S. and Marti, E. 2000. Allergen-specific IgE levels against crude mould and storage mite extracts and recombinant mould allergens in sera from horses affected with chronic bronchitis. *Vet Immunol Immunopathol* **73**, 241–253.
- Eder, C., Curik, I., Brem, G., Cramer, R., Bodo, I., Habe, F., Lazary, S., Sölkner, J. and Marti, E. 2001. Influence of environmental and genetic factors on allergen-specific immunoglobulin E levels in sera from Lipizzan horses. *Equine Vet J* **33**, 714–720.

- Eyre, P. 1972. Equine pulmonary emphysema: a bronchopulmonary mould allergy. *Vet Rec* **91**, 134–140.
- Evans, A., Paradis, G., and O'Callaghan, M.R. 1992. Intradermal testing of horses with chronic obstructive pulmonary disease and recurrent urticaria. *Am J Vet Res* **53**, 203–208.
- Frigeri, L. and Apgar, J.R. 1999. The role of actin microfilaments in the down-regulation of the degranulation response in RBL-2H3 mast cells. *J Immunol* **162**, 2243–2250.
- Franchini, M., Gill, U., von Fellenberg, R. and Bracher, V.V. 2000. Interleukin-8 concentration and neutrophil chemotactic activity in bronchoalveolar lavage fluid of horses with chronic obstructive pulmonary disease following exposure to hay. *Am J Vet Res* **11**, 1369–1374.
- Gerber, V., Lindberg, A., Berney, C. and Robinson, N.E. 2004. Airway mucus in recurrent airway obstruction-short-term response to environmental challenge. *J Vet Intern. Med* **18**, 92–97.
- Halliwell, R.E., McGorum, B.C., Irving, P. and Dixon, P.M. 1993. Local and systemic antibody production in horses affected with chronic obstructive pulmonary disease. *Vet Immunol Immunopathol* **38**: 201–215.
- Hare, J.E., Viel, L., Conlon, P.D. and Marshall, J.S. 1999. In vitro allergen-induced degranulation of pulmonary mast cells from horses with recurrent airway obstruction (heaves). *Am J Vet Res* **60**, 841–847.
- Hellberg, W., Wilson, A.D., Mellor, P., Doherr, M.G., Torsteinsdottir, S., Zurbriggen, A., Jungi, T. and Marti, E. 2006. Equine insect bite hypersensitivity: immunoblot analysis of IgE and IgG subclass responses to *Culicoides nubeculosus* salivary gland extract. *Vet Immunol Immunopathol* **113**, 99–112.
- Horohov, D.W., Beadle, R.E., Mouch, S. and Pourciau, S.S. 2005. Temporal regulation of cytokine mRNA expression in equine recurrent airway obstruction. *Vet Immunol Immunopathol* **108**: 237–245.
- Ishizaka, K. and Ishizaka, T. 1966. Physicochemical properties of reaginic antibody. 1. Association of reaginic activity with an immunoglobulin other than gammaA- or gammaG- globulin. *J Allergy* **37**, 169–185.
- Jackson, K.A., Stott, J.L., Horohov, D.W. and Watson, J.L. 2004. IL-4 induced CD23 (FcεRII) up-regulation in equine peripheral blood mononuclear cells and pulmonary alveolar macrophages. *Vet Immunol Immunopathol* **101**, 243–250.
- Kim, T.D., Eddlestone, G.T., Mahmoud, S.F., Kuchty, J. and Fewtrell, C. 1997. Correlating Ca²⁺ responses and secretion in individual RBL-2H3 mucosal mast cells. *J Biol Chem* **272**, 31225–31229.
- Künzle, F., Gerber, V., van der Haegen, A., Wampfler, B., Straub, R. and Marti, E. 2007. IgE-bearing cells in bronchoalveolar lavage fluid and allergen-specific IgE levels in sera from RAO-affected horses. *J Vet Med A Physiol Pathol Clin Med* **54**, 40–47.
- Lavoie, J.P., Maghni, K., Desnoyers, M., Taha, R., Martin, J.G. and Hamid, Q.A. 2001. Neutrophilic airway inflammation in horses with heaves is characterized by a Th2-type cytokine profile. *Am J Respir Crit Care Med* **164**, 1410–1413.
- Lorch, G., Hillier, A., Kwochka, K.W., Saville, W.J., Kohn, C.W. and LeRoy, B.E. 2001a. Comparison of immediate intradermal test reactivity with serum IgE quantitation by use of a radioallergosorbent test and two ELISA in horses with and without atopy. *J Am Vet Med Assoc* **218**, 1314–1322.
- Lorch, G., Hillier, A., Kwochka, K.W., Saville, W.J., Kohn, C.W. and Jose-Cunilleras, E. 2001b. Results of intradermal tests in horses without atopy and horses with chronic obstructive pulmonary disease. *Am J Vet Res* **62**, 389–397.
- Marti, E. and Harwood, L. 2002. Genetics of human asthma and equine recurrent airway obstruction: can these two disease be compared?. *Pferdheikunde* **18**, 587–593.
- Matthews, A.G., Imlah, P., and McPherson, E.A., 1983. A reagin-like antibody in horse serum: 1. Occurrence and some biological properties. *Vet Res Com.* **6**, 13–23.
- McGorum, B.C., Dixon, P.M. and Halliwell, R.E. 1993. Evaluation of intradermal mould antigen in the diagnosis of equine chronic obstructive pulmonary disease. *Equine Vet J* **25**, 273–275.
- McPherson, E.A., Lawson, G.H., Murphy, J.R., Nicholson, J.M., Breeze, R.G. and Pirie, H.M. 1979. Chronic obstructive pulmonary disease (COPD) in horses: aetiological studies: responses to intradermal and inhalation antigenic challenge. *Equine Vet J* **11**: 159–166.
- Metzger, H. 1992. The receptor with high affinity for IgE. *Immunol Rev* **125**, 37–48.
- Morgan, E.E., Miller, W.H Jr., and Wagner, B. 2007. A comparison of intradermal testing and detection of allergen-specific immunoglobulin E in serum by enzyme-linked immunosorbent assay in horses affected with skin hypersensitivity. *Vet Immunol Immunopathol* **120**, 160–167.
- Moran, G., Araya, O., Orloff, A. and Folch, H. 2009. Cytologic bronchoalveolar lavage findings and humoral immune response against *Aspergillus fumigatus* in Chilotes horses with Recurrent Airway Obstructions “Heaves”. *Arch Med Vet* **41**, 83–88.
- Prouvost-Danon, A., Binaghi, R.A. and Abadie, A. 1977. Effect of heating at 56 degrees C on mouse IgE antibodies. *Immunochemistry* **14**, 81–84.
- Robinson, N.E., Olszewski, M.A. and Boehler, D. 2000. Relationship between clinical signs and lung function in horses with recurrent airway obstruction (heaves) during a bronchodilator trial. *Equine Vet J* **32**: 393–400.
- Schmallenbach, K.H., Rahman, I., Sasse, H.H., Dixon, P.M., Halliwell, R.E., McGorum, B.C. and Miller, H. R. 1998. Studies on pulmonary and systemic *Aspergillus Fumigatus*-specific IgE and IgG antibodies in

- horses affected with chronic obstructive pulmonary disease (COPD). *Vet Immunol Immunopathol* **66**: 245–256.
- Scharenberg, A. M. and Kinet, J.P. 1995. Early events in mast cell signal transduction. *Chem Immunol* **61**, 72–87
- Schatzmann, U. and Gerber, H. 1972. Untersuchungen zur Aetiologie chronischer Lungenkrankheiten des Pferdes. *Zbl Vet Med A* **19**, 89–101.
- Tahon, L., Baselgia, S., Gerber, V., Doherr, M.G., Straub, R., Robinson, N.E. and Marti, E. 2009. *In vitro* allergy test compared to intradermal testing in horses with recurrent airway obstruction. *Vet Immunol Immunopathol* **127**, 85–93
- Tkaczyk, C. and Gilfillan, A. 2001. FcεRI-Dependent Signaling Pathways in Human Mast Cells. *Clin Immunol* **99**: 198–210.
- van der Haegen, A., Künzle, F., Gerber, V., Welle, M., Robinson, N.E. and Marti, E. 2005. Mast cells and IgE-bearing cells in lungs of RAO-affected horses. *Vet Immunol Immunopathol* **15**: 325–334
- Wagner, B., Radbruch, A., Rohwer, J. and Leibold, W. 2003. Monoclonal anti-equine IgE antibodies with specificity for different epitopes on immunoglobulin heavy chain of native IgE. *Vet. Immunol Immunopathol* **92**: 45–60.
- Wong, D.M., Buechner-Maxwell, V.A., Manning, T.O. and Ward, D.L. 2005. Comparison of results for intradermal testing between clinically normal horses and horses affected with recurrent airway obstruction. *Am J Vet Res* **66**: 1348–1355.