

Influence of environmental and genetic factors on allergen-specific immunoglobulin-E levels in sera from Lipizzan horses

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Summary

To investigate whether allergen-specific IgE production is influenced by environmental and genetic factors, IgE levels against 2 mould extracts (*Alternaria alternata* [Alt a] and *Aspergillus fumigatus* [Asp f]) and against recombinant (r) rAlt a 1, rAsp f 7 and rAsp f 8 were determined by ELISA in sera from 448 Lipizzan horses living in 6 studfarms.

Statistical evaluation showed a significant effect of studfarm-specific environment on IgE levels against the different allergens, but genetic factors also influenced allergen-specific IgE production: an heritability of 0.33 was found for IgE levels against the 2 mould extracts and of 0.21 for rAsp f 8-specific IgE. Heritability estimates for rAlt a 1- and rAsp f 7-specific IgE were negligible.

Investigations for a possible association between Major Histocompatibility Complex (MHC) class I antigens and specific IgE levels were carried out. The most consistent significant association was found between the equine leucocyte antigen (ELA) A8 and undetectable IgE titres against rAsp f 7 and rAsp f 8. Significant ELA associations were also demonstrated between ELAA1 and higher specific IgE levels, between ELA A14 and lower IgE levels against the mould extracts and in one studfarm between ELA Be27 and lower *Aspergillus*-specific IgE levels.

Introduction

Atopic disorders represent a subgroup of allergic diseases with a genetic predisposition to generate immunoglobulin E (IgE) against certain otherwise innocuous environmental allergens. However, a prerequisite for the induction of allergen-specific IgE is the exposure to the respective allergens. Production of specific IgE is initiated by uptake and presentation of allergens by major

histocompatibility complex (MHC) class II positive cells to allergen-specific helper T lymphocytes that then instruct B lymphocytes to produce allergen-specific antibodies. Allergen-specific IgE antibodies bind to the high affinity receptors present on mast cells (MCs)/basophils. Subsequent IgE cross-linking mediated by allergen exposure results in release of various inflammatory mediators, such as histamine and leukotrienes, and cytokines that trigger the 'allergic cascade' (Lichtenstein 1993).

In man, different studies have shown that the ability to produce high or low IgE responses is inherited (Meyers 1993) and, more recently, association or linkage of total serum IgE level with a number of different genes, such as the IgE receptor gene and cytokine genes have been demonstrated (Barnes and Marsh 1998). Other studies have shown that, additionally to genetic factors which influence a general predisposition for high IgE levels, there are also associations between MHC class II antigens/T-cell receptor and the specific IgE response against single, well-defined allergenic molecules (Barnes and Marsh 1998).

The term of 'atopy' has never been used in the horse for diseases caused by hypersensitivity reactions to environmental allergens, like sweet-itch or chronic bronchitis (CB), although a genetic predisposition has been demonstrated for these 2 conditions (Marti *et al.* 1991, 1992). Proof that these diseases are due to IgE-mediated hypersensitivity reactions is still missing, although there is growing evidence that IgE plays an important role in the pathogenesis of sweet-itch, an allergic dermatitis caused by bites of insects, mainly *Culicoides* spp. (Fadok and Foil 1990; Marti *et al.* 1999; van der Haegen *et al.* 2001). The pathogenesis of CB, a hypersensitivity reaction to moulds present in hay and straw (Robinson *et al.* 1996) is less clear, although a number of studies suggest an involvement of IgE in the development of this disease, also called chronic obstructive pulmonary disease (COPD). Halliwell *et al.* (1993) and Schmallenbach *et al.* (1998) have shown that horses with COPD have higher IgE levels against *Micropolyspora faeni*, *Aspergillus fumigatus* and a recombinant (r) *Aspergillus fumigatus* allergen (rAsp f I/a) in bronchoalveolar lavage fluid (BALF) compared to

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TABLE 1: Distribution of the 448 Lipizzan horses in 6 studfarms. The number of stallions and mares, as well as the age of the horses and the allele frequency of equine leucocyte antigens (ELA) class I are shown for each studfarm. ELA typing was successfully performed in 427 of the 448 horses. Values in bold denote alleles that have been used in the gene substitution models

	D	Studfarms					Total
		L	M	Sz	T	V	
n	56	59	62	76	42	153	448
Sex (stallions/mares; n)	15/41	19/40	15/47	8/68	5/37	72/81	134/314
Age (years); median (range)	7 (4–21)	10 (4–24)	10 (3–21)	9 (3–23)	11 (4–22)	10 (4–34)	10 (3–34)
No. horses tested for ELAclass I	53	58	52	76	35	153	427
ELAclass I allele frequency for:							
A1	0.085	0.138	0.106	0.105	0.100	0.190	0.137
A2	0.066	0.034	0.106	0.039	0.171	0.062	0.069
A3	0	0.026	0.010	0.007	0.029	0.095	0.042
A4	0.038	0.060	0	0	0	0.101	0.049
A7	0.028	0.155	0.019	0.158	0.029	0	0.057
A8	0.028	0.043	0	0.178	0	0.007	0.043
A10	0	0	0	0	0	0.003	0.001
W11	0	0	0.029	0	0	0.010	0.007
A14	0.047	0.181	0.019	0.033	0.086	0.033	0.057
A16	0.038	0.034	0.221	0.059	0.214	0.147	0.117
A17	0	0	0	0.013	0	0.013	0.007
A19	0.500	0.026	0.481	0.355	0.229	0.101	0.242
A20	0	0.009	0	0	0	0	0.001
Be27	0.170	0.293	0	0.046	0.114	0.092	0.111
Be28	0	0	0	0	0	0.052	0.019
Uninformative	0	0	0.010	0.007	0.029	0.095	0.039

control horses. In both studies, no significant differences between COPD and healthy horses could be demonstrated when IgE levels against these allergens were determined in serum. Similarly to these results, Eder *et al.* (2000) could not find significant differences in serum IgE levels against mould extracts between CB-affected and healthy horses. However, they found higher IgE levels in serum of CB-affected horses compared to controls using highly pure *rAspergillus fumigatus* (rAsp f 7, rAsp f 8) and *rAlternaria alternata* (rAlt a 1) allergens. Histamine release assays with peripheral blood leucocytes (Dirschel *et al.* 1993) or mast cells from BALF (Hare *et al.* 1999) also suggest an involvement of IgE mediated reactions in COPD. However, it remains to be proven if the characteristic increase of neutrophils in BALF after exposure of COPD-affected horses with mouldy hay (Derksen *et al.* 1985) is a consequence of IgE-mediated reactions and/or which other immunological mechanisms are involved in the pathogenesis of COPD.

The aim of the present study was to analyse if studfarm (i.e. environment) and age have an impact on allergen-specific IgE levels in the horse. We also wanted to investigate if genetic factors influence the specific IgE response and if an association between the equine leucocyte antigens (ELA) and IgE levels against mould allergens, in particular against well-defined recombinant allergens, could be detected. For that purpose serum IgE against mould allergen extracts and recombinant mould allergens was determined in sera from 448 mature horses belonging to the same breed but living in 6 different studfarms.

Materials and methods

Horses

Lipizzan horses (n = 448), almost exclusively broodmares and stallions, belonging to 6 studfarms from 6 different countries (Croatia: studfarm D; Slovenia: studfarm L; Italy: studfarm M;

Hungary: studfarm SZ; Slovakia: studfarm T; Austria: studfarm V) were included in this study. Distribution, age and sex of the animals are shown in Table 1. All tested horses were age >3 years. The mares were kept loose in group housing stables during the winter and during the summer at pasture and in stables except the mares from studfarm M, which were stabled only during the winter and spent the rest of the year at pasture. The stallions were held in individual loose boxes in another stable than the mares from the respective studfarms but were fed the same hay. The stallions from studfarms SZ and D were kept in stalls and ventilation of their stables was rather poor. Stallions from studfarm M were at pasture from spring to summer, each sire with its herd of mares. All horses were fed hay in winter and also to some extent in summer, additionally to pasture.

Blood samples

Blood was collected from the jugular vein into sodium heparin containing vacutainers (Vacuette)¹ for the serological MHC typing and with vacutainers containing serum clot activator¹ for preparation of serum. Serum was frozen in aliquots within 24 h after blood sampling and then stored at -70°C until assayed.

Serological MHC class I typing

Alloantisera detecting 17 internationally recognised (A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, W11, A14, A15, A16, A17, A18, A19 and A20) and 4 locally defined (Be22, Be25, Be27 and Be108) ELA-A locus specificities were tested in a classic 2-step microcytotoxicity test in Terasaki plates according to Lazary *et al.* (1980, 1988). Furthermore, in studfarm V a new locally-defined ELA-A allele (Be28) was recognised. In some horses only one defined ELA class I allele was found; however, reactions with not yet defined alloantisera suggest that these horses were not homozygotes but carry yet

TABLE 2: Percentage of Lippizzan horses with detectable serum IgE against recombinant *Alternaria alternata* 1 (rAlt a 1) and against recombinant *Aspergillus fumigatus* 7 and 8 (rAsp f 7 and rAsp f 8) allergens in each studfarm

Studfarm	n	rAlt a 1	rAsp f 7	rAsp f 8
D	55	54%	22%	9%
L	59	5%	12%	12%
M	63	11%	13%	3%
Sz	76	34%	37%	22%
T	42	45%	14%	2%
V	153	17%	19%	20%
Total	448	25%	20%	14%

unidentified alleles. These alleles were regarded as uninformative and are mentioned as such in Table 1. Allele frequencies (Table 1) were calculated by gene counting for all Lippizzans taken together and for each studfarm separately. Pairwise comparisons of the allele frequencies between the studfarms were carried out with Genepop, version 3.2a (Raymond and Rousset 1995a). An unbiased estimate of the P value of the probability test was performed, as described by Raymond and Rousset (1995b). All pairwise comparisons were highly significant, even when Bonferroni correction for multiple comparisons was applied.

Determination of allergen-specific IgE in horse serum

Allergen-specific serum IgE levels were determined as described previously (Eder *et al.* 2000). Briefly, microtitre² plates were coated overnight with *Alternaria alternata*³ (Alt a) or *Aspergillus fumigatus*³ (Asp f) extracts or with rAlt a 1⁴, rAsp f 7 or rAsp f 8 (Crameri 1998). Nonspecific binding sites were blocked with 0.2% equine serum albumin. Horse sera were then added at a dilution of 1:3 or 1:6, depending on the allergen used and 4 serial 1:1.5 dilutions were made in the plate. After incubation and washing, a chicken antibody specific for horse IgE (Marti *et al.* 1997) was added for 2 h. After washing, plates were incubated with an alkaline phosphatase-labelled goat anti-chicken IgG⁵. ELISA plates were subsequently developed with p-nitrophenyl phosphate⁶ in diethanolamine⁷ buffer. Absorbance readings were measured at 405 nm with a Molecular Devices Reader⁸ and converted to relative ELISA units (REU). For that purpose, an equine serum positive for the tested allergen was used as reference serum and assigned a value of 100 REU. Serial dilutions of the reference serum were used to generate a standard curve and the test sample results were calculated from the curve using an ELISA software programme. Serum from one horse was used to monitor interassay variations.

Statistical methods

Influence of studfarm, sex and age on allergen-specific IgE levels: The effects of studfarm, sex and age on allergen-specific IgE levels were analysed by (linear model) least-squares (LS) analysis using the GLM procedure (Anon 1996) for *Alternaria* and *Aspergillus*-specific IgE levels. A logistic regression GENMOD procedure (Anon 1996) was performed for rAlt a 1, rAsp f 7 and rAsp f 8-specific IgE, treating them as binary

variables (detectable or undetectable IgE levels). The following procedure was applied in all cases. First, for each dependent variable we tested a model that included, as independent variables, the effects of stud, sex and age (as a continuous variable) as well as all possible 2-way interactions among these variables. Interaction and main effects were subsequently omitted from the model if they were nonsignificant.

Estimation of heritabilities: For IgE against the r-allergens, conversely to the environmental and gene substitution models, quantitative and not binary measurements were used. Heritabilities for IgE titres against the various allergens were estimated by adding the random additive genetic effect of animal to the environmental models described above. This effect was assumed to be normally distributed with variance-covariance matrix $A\sigma_a^2$, where A is the relationship matrix and σ_a^2 is the additive genetic variance. The residual was assumed to be normally distributed with mean = 0 and variance = $I\sigma_e^2$ where I is the identity matrix and σ_e^2 is the residual variance. Restricted maximum likelihood (REML) procedures were used to estimate σ_a^2 and σ_e^2 and the (narrow sense) heritability calculated as $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$. Analyses were performed with the programme package VCE Version 4.2.5. (Groeneveld 1998). It has to be mentioned that one assumption for the validity of REML analyses is the normality of the residuals, which was not given for the reaction against the recombinant allergens. Results for those traits have therefore to be viewed with caution.

Gene substitution models: To test the effects of ELA class I genotypes on allergen-specific IgE levels, ELA alleles were included in the environmental models used above to analyse influences of studfarm, sex and age. If different ELA class I genotypes were considered in the model as fixed effects, both, additive and dominance effects on allergen-specific IgE levels could have been analysed. As ELA class I is an extremely polymorphic locus, a much larger data set would have been required to obtain statistically acceptable numbers in all genotype classes. Therefore, we were limited to the estimation of additive effects with a gene substitution model. In each analysis, overall or within studfarms, the rare and undefined alleles were pooled and treated as single alleles (see Table 1). The effects of the different alleles on specific IgE levels were estimated by multiple regression analysis. Dependencies that occur in gene substitution model because the value of the last allele is known were removed in the same way as suggested by Østergård *et al.* (1989). The analyses were also performed for each studfarm separately.

Results

Allergen-specific IgE levels

IgE against *Alternaria* and *Aspergillus* extracts could be measured in all except in 5 of the 448 sera tested (Studfarm M, n = 2; SZ, n = 3). r-allergen-specific IgE was below the detection limit of the assay in many instances and measured in only 2–45% of the sera depending on the tested r-allergen and on the studfarm (Table 2). No r-allergen-specific IgE could be measured in the 5 horses with undetectable IgE against the allergen extracts.

The reproducibility of the different ELISA was high. The interplate coefficients of variation were below 12% for the IgE ELISAs against all 5 allergens.

TABLE 3: Significance levels of studfarm, sex and age effects on IgE titres against *Alternaria alternata* (Alt a) and *Aspergillus fumigatus* (Asp f) extracts and against recombinant *Alternaria alternata* 1 (rAlt a 1) and recombinant *Aspergillus fumigatus* 7 and 8 (rAsp f 7 and rAsp f 8) allergens in sera from 448 Lipizzan horses

Effects	Linear models		Logistic regression		
	Alt a.	Asp f.	rAlt a 1	rAsp f 7	rAsp f 8
Studfarm	***	***	***	***	***
Age	*	**	NS	NS	NS
Sex	NS	NS	NS	NS	NS
Studfarm *Age	***	***	NS	*	NS
Studfarm *Sex	***	***	NS	NS	NS

NS = not significant, *P≤ 0.05, **P≤ 0.01 and ***P≤ 0.001

Influence of studfarm, age and sex on allergen-specific IgE levels

Highly significant (P = 0.001) studfarm effects were found on serum IgE levels against all 5 tested allergens (Table 3). LS means of *Alternaria*-specific IgE ranged from 56 in studfarm T to 86 in studfarm L and LS means of *Aspergillus*-specific IgE ranged from 74 in studfarm T to 91 in studfarm M (Table 4). When data from all 448 horses were analysed together, no significant sex effects could be shown but highly significant interactions between studfarm and sex (Table 3) were demonstrated, i.e. sex effects were different from studfarm to studfarm (Table 4). A similar phenomenon was observed with age; there was a general significant tendency for increasing IgE levels with age (Table 4), but no such effect was found in studfarm D and V and IgE levels were even significantly decreasing with age in studfarm T. The interaction between studfarm and age was therefore also highly significant (Table 3). No significant effects of sex and age on r-allergen-specific IgE could be demonstrated, probably due to the high number of horses with undetectable IgE levels against the r-allergens.

Heritabilities of allergen-specific serum IgE

Pedigrees were available often up to 25 generations. Table 5 shows heritabilities of 0.33 for *Alternaria*-specific IgE and of 0.34 for *Aspergillus*-specific IgE, i.e. one third of the phenotypic variation in IgE levels can be explained by polygenetic additive effects. A somewhat lower heritability (0.21) was found for IgE levels against rAsp f 8, while heritability estimates for the other

2 r-allergens were negligible (Table 5).

Association between ELA and allergen-specific IgE levels

There were significant positive associations between ELA A1 and IgE levels against *Alternaria* extract in studfarm D (P≤ 0.001) and between ELA A1 and *Aspergillus* extract-specific IgE in studfarms D (P≤ 0.01) and L (P≤ 0.05; Table 7). The latter association was also significant (P≤ 0.05) when data of the 427 horses from all studfarms were analysed together (Table 6). A significant (P≤ 0.05) positive association between rAsp f 7-specific IgE levels and ELA A1 (Table 6) was found when the analysis was carried out in the 427 ELA-typed Lipizzans from the 6 studfarms taken together. This association was, however, not significant when the analysis was performed within studfarms, although in each more ELA A1 carrying horses had detectable rAsp f 7-specific IgE than horses without ELA A1 (data not shown).

ELA A8 displayed a significant (P<0.01) negative association with rAlt a 1-, rAsp f 7- and rAsp f 8-specific IgE titres when the analysis was performed with the 427 Lipizzans taken together (Table 6). This association was confirmed for rAsp f 7 and rAsp f 8-specific IgE titres when the analysis was performed within studfarm SZ (Table 7), where the majority of the ELAA8 carrying horses lived (Table 1).

Dividing r-allergen-specific IgE titres into detectable and undetectable IgE levels illustrates the negative association between ELAA8 and IgE levels against r-allergens:

Of the 25 horses from studfarm SZ that carried ELA A8, only 16% (4 horses) had detectable IgE levels against rAsp f 7 while 47% (24 horses) of the 51 horses without this ELA had detectable IgE against rAsp f 7. A similar phenomenon was observed for rAsp f 8-specific IgE titres: only 8% (n = 2) of the 25 horses that carried ELAA8 had detectable IgE levels against this r-allergen while 29% (n = 15) of the 51 horses without A8 had measurable IgE against rAsp f 8. The 10 additional horses carrying ELA A8, but living in the other studfarms had undetectable IgE levels against rAsp f 7 and rAsp f 8.

The negative association between rAlt a 1-specific IgE and ELAA8 was not significant within studfarm SZ (P = 0.07) but became significant when the 427 horses from all studfarms were analysed together (Table 6); 16% (n = 4) of the 25 horses from studfarm SZ carrying ELAA8 had measurable IgE titres against rAlt a 1, whereas 43% (n = 22) of the 51 horses from studfarm SZ without this ELA had detectable IgE against rAlt a 1. Only one of the 10 horses carrying A8, living in the other studfarms had detectable IgE against rAlt a 1.

TABLE 4: Effects of studfarm, sex and age on serum IgE levels (relative ELISAunits, REU) against mould extracts (*Alternaria alternata* and *Aspergillus fumigatus*) in the 448 Lipizzan horses

Horses from studfarms	REU (LS means)	<i>Alternaria a.</i>		REU (LS means)	<i>Aspergillus f.</i>	
		Stallions/mares	Age		Stallions/mares	Age
		REU (LS means)	REU/year (partial regression coefficient)		REU (LS means)	REU/year (partial regression coefficient)
All together	75	75/76	0.58*	84	83/85	1.03**
D	66	67/66	0.70 NS	83	82/83	1.09 NS
L	86	97/75	2.88**	89	96/81	5.83***
M	83	63/103	3.08***	91	68/115	3.83***
Sz	76	83/68	0.65*	77	83/72	1.83*
T	56	58/57	-0.78**	74	81/67	-0.57**
V	85	85/84	-0.10 NS	90	88/91	0.30 NS

NS = not significant, *P≤ 0.05, **P≤ 0.01 and ***P≤ 0.001; LS = least squares.

TABLE 5: Estimates of additive genetic (σ_a^2) and residual (σ_e^2) variances, of heritability (h^2) and standard error of heritability (S_{h^2}) on specific IgE levels against *Alternaria alternata* and *Aspergillus fumigatus* extracts as well as against recombinant (r) *Alternaria* (rAlt a 1) and *Aspergillus* (rAsp f 7 and rAsp f 8) allergens in sera from 448 Lipizzan horses

IgE specific for	σ_a^2	σ_e^2	h^2	S_{h^2}
<i>Alternaria a.</i>	202.05	417.66	0.33	0.09
<i>Aspergillus f.</i>	328.49	643.52	0.34	0.10
rAlt a 1	20.73	809.05	0.03	0.06
rAsp f 7	25.68	763.21	0.03	0.06
rAsp f 8	51.66	194.39	0.21	0.08

Three horses from studfarm SZ, with no detectable serum IgE against *Aspergillus* and *Alternaria* extracts nor against the 3 r-allergens, were all carrying ELA A8 and one of them was homozygote for A8.

ELA A14 carrying horses had lower IgE titres against the mould extracts than horses without this ELA antigen (Table 6, $P \leq 0.05$). These negative associations were found in studfarm L (*Alternaria*-specific IgE, Table 7, $P < 0.05$) and when the analysis was performed with all 427 horses (*Alternaria*- and *Aspergillus*-specific IgE, $P \leq 0.05$, Table 6). ELA A14 carrying horses from studfarms L and T had also lower IgE levels against *Aspergillus* than horses without this ELA; however, the difference was not significant when the analysis was carried out within studfarms. In

studfarm D, horses carrying the ELA Be27 had lower *Alternaria*-specific IgE titres than horses without this ELA allele ($P \leq 0.05$).

Discussion

Access to 450 mature horses of the same breed but living in 6 different locations was a rare opportunity to study the effects of environmental and genetic factors on specific IgE levels. A clinical examination of the respiratory tract to identify horses suffering from CB could unfortunately not be carried out because many of the broodmares were not used to handling, rendering a thorough examination of the respiratory tract impossible without sedation. We could not, therefore, exclude infections of the respiratory tracts and did not include diagnosis as a variable in the statistical analysis. Previous studies (Robinson *et al.* 1996) suggest that at least 10% of the horses were affected with CB.

Specific IgE levels against mould extracts were determined because a previous investigation had shown that IgE against *Alternaria* and *Aspergillus* extracts can be measured in nearly all horse sera (Eder *et al.* 2000). The r-allergens were included because significantly higher IgE titres against these allergens, in particular against rAsp f 8, were found in CB-affected compared to healthy horses (Eder *et al.* 2000). Furthermore, associations with the MHC are to be expected with well-defined single allergenic molecules. Although determination of total IgE levels would have been very interesting, it was not performed because a second equine IgE-specific antibody, presently not available, would have been necessary.

TABLE 6: Significance of gene substitution effects at the ELA class I locus for specific IgE levels against *Alternaria alternata* and *Aspergillus fumigatus* extracts and against recombinant (r) *Alternaria* (rAlt a 1) and *Aspergillus* (rAsp f 7 and rAsp f 8) allergens in sera from 427 Lipizzan horses. Arrows ($\uparrow \downarrow$) show if horses carrying the tested ELAallele have significantly higher or lower IgE titres than horses not carrying this ELAallele

Allele	<i>Alternaria a.</i>	<i>Aspergillus f.</i>	rAlt a 1	rAsp f 7	rAsp f 8
A1	ns	$\uparrow^*$	ns	$\uparrow^*$	ns
A2	ns	ns	ns	ns	ns
A3	ns	ns	ns	ns	ns
A4	ns	ns	ns	ns	ns
A7	ns	ns	ns	ns	ns
A8	ns	ns	** $\downarrow$	*** $\downarrow$	** $\downarrow$
A14	* $\downarrow$	* $\downarrow$	ns	ns	ns
A16	ns	ns	ns	ns	ns
A19	ns	ns	ns	ns	ns
Be27	ns	ns	ns	ns	ns

ns = not significant, * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

TABLE 7: Significance of gene substitution effects at the ELA class I locus for specific IgE levels against *Alternaria alternata* and *Aspergillus fumigatus* extracts and against recombinant (r) *Alternaria* (rAlt a 1) and *Aspergillus* (rAsp f 7 and rAsp f 8) allergens in sera from the Lipizzans in each studfarm. Arrows ($\uparrow \downarrow$) show if horses carrying the tested ELA allele have significantly higher or lower IgE titres than horses not carrying this ELA allele. The analysis was inconclusive (-) when there were less then 6 horses with detectable IgE against the r-allergens within studfarms

Studfarm	<i>Alternaria a.</i>	<i>Aspergillus f.</i>	rAlt a 1	rAsp f 7	rAsp f 8
D	$\uparrow$ A1***	A1 $\uparrow$ **/ $\downarrow$ Be27*	NS	-	NS
L	$\downarrow$ A14*	$\uparrow$ A1*	-	NS	NS
M	NS	NS	NS	-	NS
Sz	NS	NS	NS	$\downarrow$ A8**	$\downarrow$ A8*
T	NS	NS	NS	-	NS
V	NS	NS	NS	NS	NS

NS = not significant, * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

We speculate that the studfarm effects (Tables 3 and 4) that influence specific IgE levels are due to environmental factors. It is not surprising that environment influences IgE levels in the horse, as studies with monozygotic and dizygotic twins have shown that, additionally to genetic factors, environment strongly affects IgE levels in man (Hopp *et al.* 1984). We suspect that the significant interactions between studfarm and sex are due to environmental factors: (i) the stallions were always held in another stable than the mares, (ii) the sex effects varied between studfarms (Table 3 and 4) and (iii) no significant sex effect could be demonstrated when the analysis was carried out with all 448 horses. The effect of age on specific IgE levels varied also from studfarm to studfarm (Tables 3 and 4) but IgE levels increased slightly with age (Table 4), except in studfarm T (Table 4), where older horses had lower IgE levels than younger ones. We have to keep in mind that only mature horses were included in this investigation. We do not know when sensitisation to environmental allergens begins in the horse. In man initial sensitisation occurs during infancy and early childhood (Holt *et al.* 1990).

Although the sample size of nearly 450 animals was small for estimation of heritabilities, the pedigrees were extremely long (up to 32 generations) and complete (for example, in the 20th generation 75% of ancestors were known). We consider that our data set was, therefore, acceptable for estimation of heritabilities. Furthermore, although the different studfarms were located in different countries, animals were exchanged and therefore the genetic connections between studfarms were sufficient for our study. The average coefficients of coancestry were 0.14–0.24 between studfarms and 0.24–0.30 within studfarms (data not shown). The heritability estimates of 0.33 and 0.34 found for *Alternaria* and *Aspergillus*-specific serum IgE levels, respectively, are close to those known in man; family studies revealed a heritability of total serum IgE levels of 25–79%. The mode of inheritance is still not clear and a great deal of heterogeneity between families is suggested in man (Meyers 1993). In our study, no heritability could be demonstrated for IgE against rAlt a 1 and rAsp f 7. Possible explanations could be the rather low sensitivity of the ELISA for IgE detection against the r-allergens, i.e. many sera were below the detection limit of the assay, or a low incidence of sensitisation against these single allergens in horses. Conversely, an heritability of 0.21 was found for the third r-allergen used, rAsp f 8, although many sera were also below the detection limit for this r-allergen.

Considering the function of the MHC, i.e. presentation of endogenous peptides by class I and of exogenous peptides by class II gene products, associations between MHC class II alleles and the IgE response against well-defined allergens would be expected. Such associations have been demonstrated in man, for example between HLA-D alleles and specific ragweed allergens (Marsh 1993). ELA class II alleles could not be determined in this study, because well-established methods for ELA class II typing are still lacking. However, ELA class I alleles are linked to class II alleles and associations, although weaker, can also be uncovered by class I typing.

The only ELA associations that were found with the recombinant allergens were a positive association between ELA-A1 and detectable rAsp f 7-specific IgE levels and between ELA-A8 and undetectable rAlt a 1, rAsp f 7 and rAsp f 8-specific IgE (Table 6). While the associations between ELA-A1 and rAsp f 7-specific IgE and ELA-A8 and rAlt a 1-specific IgE were

significant only when horses from all studfarms were analysed together, the negative association between ELA A8 and low IgE levels against rAsp f 7 and 8 was also significant in studfarm SZ. Most of the ELA-A8 carrying horses lived in studfarm SZ, but interestingly this studfarm had the highest percentage of horses with detectable IgE levels against rAsp f 7 and rAsp f 8 (Table 2). The uncovered negative association between ELA A8 and r-allergen-specific IgE levels was therefore certainly not due to a studfarm effect. Associations between one ELA allele and IgE levels against different r-allergens were sometimes found. This may be explained by the fact that horses, in particular CB-affected ones, often have high IgE levels against more than one r-allergen (Eder *et al.* 2000). This speculation is supported by the fact that associations between one particular ELA and IgE against different allergens were always in the same direction i.e. positive or negative with all allergens. A similar phenomenon was observed with IgE levels against the allergen extracts: associations between ELA A1 and high IgE and A14 and low IgE levels (Tables 6 and 7) were found for both allergen extracts. This is not surprising if we consider the strong positive correlation between *Alternaria* and *Aspergillus*-specific IgE levels that was described by Eder *et al.* (2000) and which is confirmed in the present study (Pearson correlation coefficient between *Alternaria* and *Aspergillus*-specific IgE = 0.9, $P \leq 0.0001$; data not shown). This finding suggests that some horses have a general tendency to produce high IgE levels against various allergens and probably also explains why we found similar heritabilities for *Alternaria* and *Aspergillus*-specific IgE. However, this result raises the question whether the associations between ELA alleles and IgE levels against the allergen extract are due to true ELA effects or to effects of other genes linked to the MHC. The presence of similar epitopes in both allergen extracts could also explain these results.

In conclusion, this study has shown that environmental factors play an important role on serum IgE levels and should be accounted for in subsequent studies on IgE titres in the horse. Further investigations are needed to analyse the effects of diagnosis and environment on allergen-specific IgE. Furthermore, genetic factors and, most probably, also the MHC or genes linked to the MHC influence the production of allergen-specific IgE. Further work, e.g. segregation studies in families and determination of ELA class II alleles, is needed to better understand the associations between MHC and allergen-specific IgE levels found in this study.

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Manufacturers' addresses

¹Greiner, Huber & Co., Reinach, Switzerland.

²Dynatech Lab. Inc., Chantilly, Virginia, USA.

³Allergopharma, Hamburg, Germany.

⁴Biomay, Linz, Austria.

⁵Kirkegaard and Perry Laboratories, Gaithersburg, Massachusetts, USA.

⁶Sigma Chemical Co., St. Louis, Missouri, USA.

⁷Fluka Chemie AG, Buchs, Switzerland.

⁸Molecular Devices, Sunnyvale, California, USA.

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