Sensitization of skin mast cells with IgE antibodies to Culicoides allergens occurs frequently in clinically healthy horses

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\textbf{ABSTRACT}

IgE antibodies are mediators of mast cell degranulation during allergic diseases. The binding of IgE to its high-affinity IgE receptor on mast cell surfaces is called “sensitization” and precedes the development of clinical allergy. Previously, intradermal injection of anti-IgE or the anti-IgG(T) antibody CVS40 induced immediate skin reactions in horses. This suggested that both IgE and IgG(T) sensitize equine skin mast cells. Here, we investigated sensitization to allergens and with IgE or IgG(T) in clinically healthy horses of different age groups. In addition, immediate skin reactions to \textit{Culicoides} were determined by intradermal testing in non-allergic horses. A total of 14\% of the young horses 1–3 years old and 38\% of the adult animals showed skin reaction to \textit{Culicoides} allergen extract. Sensitization with IgE and IgG(T) was evaluated in skin mast cells and peripheral blood basophils to determine whether sensitization with IgG(T) preceded that with IgE in young horses. Anti-IgE stimulated immediate skin reactions in 18 of 21 young horses, but only 7 of them reacted to the anti-IgG(T) antibody CVS40. The equine IgG(T) fraction is composed of IgG3 and IgG5. We used several newly developed monoclonal antibodies to IgG3 and IgG5 for intradermal testing to improve our understanding about the mast cell reaction induced by the anti-IgG(T) antibody CVS40. None of these antibodies induced a skin reaction in young or adult horses. To determine sensitization with IgE in neonates and foals at 6 and 12 weeks of age an in vitro histamine release assay was performed using peripheral blood cells. The histamine concentration released by anti-IgE stimulation from foal basophils increased between birth and 12 weeks of age, while almost no histamine release was observed after anti-IgG(T) treatment of the cells. In summary, IgE was the major immunoglobulin involved in the sensitization of mast cells and basophils in horses at various ages. IgG(T) antibodies did not play a major role in the activation of mast cells or basophils in young horses and their role in the sensitization of adult horses remains unclear. Sensitization to \textit{Culicoides} allergen in the absence of clinical disease was frequently found in horses of all age groups. Because many clinically healthy horses developed skin reactions to this allergen, sensitization results are useful to diagnose \textit{Culicoides}-induced allergy only in horses with allergic conditions.

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1. Introduction

IgE is a key molecule in type-I hypersensitivities. This form of allergic disease is characterized by an immediate onset of clinical signs after exposure to allergen. Examples
Skin hypersensitivity is a common type-I allergy in horses. *Culicoides* allergens are the most important inducers of skin hypersensitivities, as shown using intradermal (i.d.) testing with *Culicoides* extracts (Braverman, 1988; Larsen et al., 1988; Greiner et al., 1990; Anderson et al., 1993; Littlewood, 1998; Steinman et al., 2003). More recently, direct evidence for the crucial role of *Culicoides*-specific IgE antibodies as mediators of the disease was found by transferring IgE antibodies from allergic donors to clinically healthy, non-sensitized recipient horses. After IgE transfer, challenge with *Culicoides* allergen extract induced immediate skin reactions in the recipient horses (Wagner et al., 2006a).

Intradermal injections of monoclonal anti-IgE and anti-IgG(T) antibodies suggested that IgE and also IgG(T) can sensitize skin mast cells in horses (Wagner et al., 2006a). In humans, IgG can be bound to the surface of mast cells and basophils via Fc-receptors for IgG (FcγRs). Depending on the type of the FcγR, receptor-bound IgG either increased mast cell activation or provided inhibitory signals after IgG crosslinking (Miyajima et al., 1997; Ujike et al., 1999; Tkaczyk et al., 2004).

Horses generally do not develop clinical signs of skin hypersensitivity before the age of 3–4 years (Wilson et al., 2001). Skin allergies are rarely observed in younger animals, although the horses are often kept in the same environment with clinically affected, allergic individuals (Wagner et al., 2003). The recent observation that foals did not produce IgE for at least 6–9 months after birth (Wagner et al., 2006b; Marti et al., 2008) might provide some explanation for the late onset of allergic diseases in horses, despite early exposure to allergens. However, IgE can be detected in the serum and on peripheral blood leukocytes of foals for the first 8–12 weeks after birth. These IgE antibodies are solely of maternal origin and are transferred with colostrum after birth (Wagner et al., 2006b; Marti et al., 2009). The highest concentrations of maternal IgE bound to the peripheral blood cells of foals were found on days 2–5 after birth and declined constantly afterwards (Wagner et al., 2006b). In contrast to the late onset of endogenous IgE production in foals, IgG(T) production was detectable after 8–10 weeks of age (Sheoran et al., 2000; Holznagel et al., 2003). Because previous findings suggested that IgG(T) could play a role in sensitization of adult horses (Wagner et al., 2006a), we hypothesized that sensitization with IgG(T) might precede that with endogenous IgE in young horses and might influence the development of allergen-specific immune responses in the absence of IgE.

This study focused on the sensitization of skin mast cells and peripheral blood basophils to *Culicoides* allergen and with IgE and IgG(T) in clinically healthy, young and adult horses. The questions to be answered by the experiments were: (1) What is the prevalence of sensitization to *Culicoides* allergen in a population of horses without clinical signs of allergy kept in a defined environment with allergen exposure? (2) Do adult horses have higher prevalence of sensitization to *Culicoides* than younger horses? (3) Are there differences in sensitization with IgE or IgG(T) in young and adult horses? (4) Does sensitization with IgG(T) precede sensitization with IgE in young horses? (5) Does stimulation with anti-IgG(T) activate mast cells or basophils in foals and young horses?

2. Materials and methods

2.1. Horses used for intradermal testing

Intradermal testing was performed on clinically healthy horses without signs of skin disease or other allergic conditions before or during this study. The adult group was composed of 24 horses 4–18 years old (median 11 years). The horses were of mixed breeds (11 Thoroughbreds, 8 Warmbloods, and 5 ponies) and sexes (21 mares and 3 geldings). Intradermal testing was also performed on 21 young horses 1–3 years old. The group of young horses consisted of 11 Thoroughbreds, 7 ponies, 2 Quarter Horses and 1 Appaloosa, 16 of which were mares and 5 were stallions.

All horses were kept in the herd of the Baker Institute for Animal Health at Cornell University (Ithaca, NY, USA) in an environment with run-in sheds and daily access to pasture. The herd was naturally exposed to *Culicoides* midges during the summer months as indicated by one animal with *Culicoides*-induced skin hypersensitivity in the herd. This horse was kept in the experimental environment for many years and developed severe signs of clinical allergy by the end of June during both years of the study. The allergic animal was not included in this sensitization study. Skin testing on adult, healthy horses was performed in May or early June, 2006. Skin tests on young horses were performed in May and November of 2006 and 2007. All experimental procedures were approved by the Animal Care Committee of Cornell University and were in accordance with the guidelines established by the NIH.

2.2. Intradermal testing

Skin testing was performed and evaluated as described previously (Wagner et al., 2006a). Briefly, for each horse, a negative (saline) and positive control (histamine, 27.5 μg/ml), various allergen extracts and monoclonal antibodies were injected i.d. in the lateral neck. The neck area was clipped the day before skin testing was performed. The volume per i.d. injection was 100 μl for adult and 50 μl for young horses. Skin reactions were read after 20–30 min. The reactions were visible as wheals at the injection sites and were evaluated by inspection and palpation. A score (0–4) was recorded for the wheal sizes at each i.d. injection site. The score of 0 was always given for the saline control.
and the score of 4 was applied to the wheal of the histamine control. The remaining reactions were rated according to these controls for each horse. The evaluation of skin reactivity and the scoring were performed blindly by a board-certified dermatologist.

2.3. Allergens for intradermal testing

A reduced panel of 36 allergens was used for skin testing in young horses. The allergen extracts were Alfalfa, Alternaria, Ash, Aspergillus, Beech, Birch, Brome, Cocklebur, Culicoides, Dandelion, Eastern Oak, Elm, Fescue, Goldenrod, June (Kentucky blue), Kochia, Lamb's Quarter, Maple Elder, Marsh Elder, Mugwort, Orchard, Pigweed, Plantain, Poplar, Quack, Ragweed, Red Cedar, Red Top, Rye, Sorrel/Dock, Sweet Vernal, Sycamore, Timothy, Walnut, White Pine, and Wormwood (Greer Laboratories, Lenoir, NC). For skin testing in adult horses, three selected allergen extracts (cat dander, Culicoides and Quack grass) were used for the following reasons: (1) The allergic horse that was used as an indicator for allergen exposure in the herd showed consistent positive reactions to Culicoides and Quack grass extracts by i.d. testing. Because the horse had lived in the herd for many years, frequent exposure to both substances was expected for all animals in this environment; (2) Culicoides allergen was the most frequent inducer of equine skin hypersensitivity (Braverman, 1988; Larsen et al., 1988; Greener et al., 1990; Ungar-Waron et al., 1990; Anderson et al., 1993; Wilson et al., 2001; Wagner et al., 2006a, 2006b); (3) IgE antibodies to cat dander extract were common in a previous serological study performed in allergic horses (Morgan et al., 2007).

2.4. Monoclonal antibodies for intradermal testing

The following mAbs were used for skin testing: anti-IgE 134 (Wagner et al., 2003, 2006a), anti-IgG(T) (CVS38 and CVS40), anti-IgGa (CVS45), anti-IgGb (CVS39) (Sheoran et al., 1998), anti-IgG3 159-4, and anti-IgG5 clones 416-2, 429-2, 522 and 586 (Wagner et al., unpublished). The anti-IgG3 and IgG5 reagents were developed in our group using previously described recombinant equine IgG3 or IgG5 proteins (Lewis et al., 2008) for immunization of mice. The mAbs were tested for their specificity to IgG3 and/or IgG5 using the recombinant proteins and detected specifically IgG5 (clones 416-2 and 522) or both IgG3 and IgG5 (clones 429-2 and 586) (Wagner et al., unpublished). The latter recognition pattern was similar to the IgG(T) antibodies CVS38 and CVS40 which also recognized both isotypes (Wagner, 2006; Lewis et al., 2008). A mAb concentration of 50 µg/ml was used for all intradermal injections. All mAbs were murine IgG1 isotypes.

2.5. Blood samples for an in vitro degranulation assay

Peripheral blood samples were obtained from foals at 12–96h after birth or at 6 or 12 weeks of age. All animals were clinically healthy throughout the sampling period. Foals were born in spring 2007 and kept in the same environment at the Equine Park at Cornell University. Samples from 12 adult, unrelated horses were also included in the degranulation assay. Blood samples were obtained in heparinized tubes and processed for the in vitro degranulation assay as previously described (Wagner et al., 2008).

2.6. In vitro degranulation assay and histamine ELISA

To provoke histamine release from blood cells, anti-IgE 134, anti-IgG(T) (CVS40), and anti-IgGa (CVS45) mAbs were used in a concentration of 5 µg/ml in Tyrodes/BSA buffer (135 mM NaCl, 5 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 5.6 mM glucose, 20 mM hepes, pH 7.4, containing 1% (w/v) BSA). Negative controls were obtained by incubation of the cells with Tyrodes/BSA alone, and positive controls by boiling the cells in Tyrodes/BSA for 5 min. The supernatants from the boiled samples were used to determine the maximal histamine release from the cells. The histamine concentration was determined in the supernatants of all samples using the Histamine EIA kit (Immuno Biological Laboratories, Inc., Minneapolis, MN). Allergen extracts have previously been reported to be irritating and histamine has been found in Culicoides extract (Wagner et al., 2008). To determine if certain allergen extracts used in intradermal testing contained detectable histamine concentrations that might have induced positive reactions by itself and independent of the respective allergen, we also measured the histamine concentrations in all allergen extracts using the Histamine EIA kit.

2.7. Statistical analysis

For comparing sensitization results between young and adult horses a Mann–Whitney test was performed. A Spearman's rank correlation was used to calculate whether the age of adult horses and the skin testing result to Culicoides allergen extract were associated. The immediate skin testing results after injection of anti-IgE or anti-IgG antibodies between different age groups were analyzed by Mann–Whitney test. A Kruskal–Wallis test followed by a Dunn's test for multiple comparisons against the adult group was used to compare the histamine release from foals (birth, 6 and 12 weeks) and adult horses for different stimuli. Most statistical calculations were performed using the GraphPad Prism program, Version 5.01. The 95% confidence intervals (CI) on prevalences and incidences were calculated in Statistix 9 (2008, Analytical Software, Tallahassee, FL). We used 2-sided α = 0.05 for significance and did not make additional adjustments for multiplicity.

3. Results

3.1. Sensitization of skin mast cells to allergen extracts in young and adult, clinical healthy horses

The prevalence of sensitization to allergen extracts was analyzed by i.d. testing in clinically healthy horses that lived in an environment with natural exposure to Culicoides midges. In young horses, the most frequent reaction was observed to Culicoides allergen extract (14%). The young horses showed a very low reactivity of overall 2% to the remaining 35 allergens (Table 1). In all 21 horses,
a single reaction was observed to Ash, Aspergillus, Brome, Eastern Oak, Marsh Elder, Quack, Ragweed, Walnut and Wormwood. Two reactions each were found to Alfalfa and Plantain.

The adult horses were tested with three selected allergen extracts (cat dander, Culicoides and Quack grass) and the controls (Fig. 1). A total of 38% of the clinically healthy, adult horses showed sensitization to Culicoides extract with a score of 2 or higher (Table 2). Only 17% of the horses reacted to Quack grass extract, and none to cat dander extract. The adult horses had a higher prevalence of sensitization to Culicoides than the young horses (p = 0.047; Fig. 2A), but sensitization to Quack grass extract did not differ (p = 0.43; Fig. 2B). Sensitization to Culicoides

### Table 1
Immediate skin reactions (30 min) to monoclonal anti-IgE and anti-IgG antibodies and to allergen extracts in 21 clinically healthy horses 1–3 years old.

<table>
<thead>
<tr>
<th>N (horses)</th>
<th>Number of reactions</th>
<th>Positive reactions</th>
<th>Score: median (min/max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st year</td>
<td>2nd year</td>
<td>3rd year</td>
<td>Total</td>
</tr>
<tr>
<td>Saline</td>
<td>5/5</td>
<td>0/7</td>
<td>0/9</td>
</tr>
<tr>
<td>Histamine</td>
<td>5/5</td>
<td>7/7</td>
<td>9/9</td>
</tr>
<tr>
<td>Anti-IgE</td>
<td>4/5</td>
<td>6/7</td>
<td>8/9</td>
</tr>
<tr>
<td>Anti-IgG(T) (CVS40)</td>
<td>1/5</td>
<td>3/7</td>
<td>3/9</td>
</tr>
<tr>
<td>Anti-IgG(T) (CVS38)</td>
<td>0/5</td>
<td>0/7</td>
<td>0/9</td>
</tr>
<tr>
<td>Anti-IgGa (CVS45)</td>
<td>0/5</td>
<td>1/7</td>
<td>0/9</td>
</tr>
<tr>
<td>Anti-IgGb (CVS39)</td>
<td>0/5</td>
<td>2/7</td>
<td>1/9</td>
</tr>
<tr>
<td>Culicoides extract</td>
<td>1/5</td>
<td>1/7</td>
<td>1/9</td>
</tr>
<tr>
<td>Other allergen extracts (35 per horse)</td>
<td>4/175</td>
<td>6/245</td>
<td>4/315</td>
</tr>
</tbody>
</table>

### Table 2
Immediate skin reactions to selected allergen extracts (Culicoides, Quack grass and cat dander), and monoclonal antibodies to IgE and IgG(T) in adult, clinically healthy horses 30 min after i.d. injection.

<table>
<thead>
<tr>
<th>N (horses)</th>
<th>Number of reactions</th>
<th>Positive reactions</th>
<th>Score: median (min/max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>24</td>
<td>0/24</td>
<td>0</td>
</tr>
<tr>
<td>Histamine</td>
<td>24</td>
<td>24/24</td>
<td>100</td>
</tr>
<tr>
<td>Anti-IgE</td>
<td>10</td>
<td>10/10</td>
<td>100</td>
</tr>
<tr>
<td>Anti-IgG(T) (CVS40)</td>
<td>10</td>
<td>9/10</td>
<td>90</td>
</tr>
<tr>
<td>Cat dander extract</td>
<td>18</td>
<td>0/18</td>
<td>0</td>
</tr>
<tr>
<td>Quack grass extract</td>
<td>24</td>
<td>4/24</td>
<td>17</td>
</tr>
<tr>
<td>Culicoides extract</td>
<td>24</td>
<td>9/24</td>
<td>38</td>
</tr>
</tbody>
</table>
was found throughout the group of adult horses and was not age-dependent (Fig. 2C).

3.2. Histamine concentrations in allergen extracts

Histamine was detected in 5 out of the 36 extracts used for skin testing in young horses and also in the cat dander extract (Table 3). The final histamine concentration injected with these extracts during skin testing was at least 1800-fold lower than the histamine concentration of the positive control. Skin testing using various concentrations of histamine was performed on four of the adult horses to identify the lowest histamine concentration that still induced a positive reaction and to assist in interpreting the influence of the histamine in some of the allergen extracts on the intradermal testing results. The histamine concentration used for the positive control in skin testing was 27,500 ng/ml. This concentration and also a histamine injection of 2750 ng/ml induced clearly positive reactions in all four horses (Fig. 3). At histamine concentrations of 27.5 ng/ml or lower immediate skin reactions were not observed. Because the histamine concentrations injected with the allergen extracts, including *Culicoides* extract, ranged between 3.8 and 15.1 ng/ml, we concluded that the histamine content of the allergen extracts alone was not sufficient to induce an immediate skin reaction in horses and that positive skin reactions resulted at least partially from *Culicoides* allergen. Thus, the immediate skin reactions observed after intradermal injection of *Culicoides* extract in clinically healthy horses were likely to represent true sensitization of skin mast cells to *Culicoides* allergen.

![Fig. 2](image-url) Immediate skin reactions to *Culicoides* (A) and Quack grass (B) allergen extracts in clinically healthy horses. Skin reactions were scored 30 min after i.d. injection and were compared by Mann–Whitney test between 21 young (1–3 years old) and 24 adult horses (>4 years old) living in the same environment. The bars indicate the median score per group. (C) Immediate skin reactions to *Culicoides* allergen extract of individual adult horses between 4 and 18 years old (rsp = –0.26, p = 0.21).

![Fig. 3](image-url) Different concentrations of histamine were injected into the skin of four adult horses. The scores of the immediate skin reactions for each horse and histamine concentration are shown. The histamine concentration of 27,500 ng/ml corresponded to that of the positive control used for all skin testing experiments in this study.

![Table 3](image-url) Histamine concentrations (ng/ml) in various allergen extracts were detected in 6 out of 37 extracts by ELISA.

<table>
<thead>
<tr>
<th>Allergen extract</th>
<th>Cat dander</th>
<th>Culicoides</th>
<th>Maple Elder</th>
<th>Marsh Elder</th>
<th>Quack grass</th>
<th>Wormwood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine concentration (undiluted extract)</td>
<td>302</td>
<td>165</td>
<td>136</td>
<td>76</td>
<td>172</td>
<td>184</td>
</tr>
<tr>
<td>Allergen dilution for i.d. testing</td>
<td>1:20</td>
<td>1:20</td>
<td>1:20</td>
<td>1:20</td>
<td>1:20</td>
<td>1:20</td>
</tr>
<tr>
<td>Final histamine concentration used for i.d. testing</td>
<td>15.1</td>
<td>8.25</td>
<td>6.8</td>
<td>3.8</td>
<td>8.6</td>
<td>9.2</td>
</tr>
</tbody>
</table>
3.3 Sensitization of skin mast cells with IgE in clinically healthy young and adult horses

Anti-IgE induced an immediate skin reaction with a score of ≥2 in 86% of the i.d. injection sites in young horses (Table 1). The same anti-IgE reagent induced an immediate skin reaction in each of the ten adult horses tested (Table 2). Although the median score of the anti-IgE-mediated immediate skin reactions increased from 2 (horses 1 and 2 old) to 3 (horses 3 years old and adults) with age, the reactions between young and adult horses were not significantly different (Fig. 4).

3.4 Skin reactivity to anti-IgG(T) antibodies in clinically healthy young and adult horses

We previously showed that the anti-IgG(T) antibody CVS40 induced immediate skin reactions in adult horses. Here, we expanded the testing by using various antibodies and found that immediate skin reactions to anti-IgG(T) depended on the antibody clone used for injection. While CVS40 injection induced a skin reaction in one-third of the young horses, CVS38 did not result in any skin reaction (Table 1). In addition, we performed skin testing on seven of the young horses (1–2 years of age) using one mAb to equine IgG3, two mAbs to equine IgG5, and two new mAbs to IgG3 and IgG5. None of these antibodies induced an immediate skin reaction in any of the horses.

Ten of the adult horses were tested with the anti-IgG(T) antibody CVS40. In response to CVS40 injection, 9 out of 10 adult horses developed an immediate skin reaction (Table 2). Although the reaction to CVS40 confirmed previously results obtained by i.d. injection of this antibody, skin reactions were not induced by the injected CVS38 or any of the anti-IgG3 or anti-IgG5 antibodies into the skin of adult horses (data not shown).

3.5 Antibody mediated in vitro degranulation from basophils of foals and adult horses

Intradermal testing is difficult to perform in neonates and young foals. Sensitization of peripheral blood basophils can also be tested by in vitro assays. Basophils have a frequency of 0.5–1% in peripheral white blood cells. They are equipped with high-affinity IgE receptors, are sensitized with IgE and release histamine in response to stimulation similar to mast cells (Langner et al., 2008; Wagner et al., 2008). A degranulation assay was used to test for anti-IgE and anti-IgG(T) (CVS40) mediated histamine release from basophils of foals and adult horses.

The histamine release induced by stimulation of the cells with anti-IgE was significantly lower at birth, 6 weeks and 12 weeks of age compared to cells from adult horses (Fig. 5A). Basophils from foals at birth and 6 weeks of age also contained significantly less histamine than basophils from adult horses (Fig. 5B). After stimulation with anti-IgG(T), low concentrations of histamine were detected in 20% of the samples from foals at birth and 6 weeks of age, in 29% of the foals at 12 weeks of age, and in 67% of the adult horses. Overall, the median histamine concentrations induced by anti-IgG(T) were lower than those induced by anti-IgE in all age groups (Table 4). In adult
horses, anti-IgG(T) stimulated an increased histamine release compared to foals \((p < 0.05\) at all time points). Anti-IgGa served as a negative control and a histamine release induced by anti-IgGa was not detectable for most foals and adult horses. No significant differences between the histamine release induced by the two anti-IgG antibodies were found for the foals or for the adult horses.

4. Discussion

Sensitization of mast cells and basophils with IgE precedes degranulation and the development of clinical signs of allergy (Ravetch and Kinet, 1991). Intradermal skin testing and in vitro degranulation assays with allergens are based on the principle of mast cell or basophil sensitization and are used in the diagnosis of allergies to identify allergens responsible for the disease. Nevertheless, sensitization to a particular allergen does not necessarily mean that the individual is allergic or will develop clinical signs of allergy. Previous reports in horses found that clinically healthy animals were sometimes sensitized to allergens. Sensitization in healthy control horses was identified by i.d. testing (Lorch et al., 2001a,b; Kolm-Stark and Wagner, 2002; Lebis et al., 2002) or by performing an in vitro sensitization assay (Wagner et al., 2008). Here, we investigated the sensitization of skin mast cells to Culicoides allergen in clinically healthy horses kept in a single environment. More than one-third of the adult horses were sensitized to Culicoides extract. We concluded that exposed horses frequently become sensitized to Culicoides allergen without showing clinical signs of allergy.

The prevalence of sensitization to Culicoides allergen was lower in young horses (14%) than in adult horses (38%). This is in agreement with the observation that skin hypersensitivity usually does not develop before 3–4 years of age (Wilson et al., 2001). If the exposure of all horses to Culicoides allergen in the same environment is considered as equal, two explanations could be offered for the higher prevalence of sensitization in healthy adult horses.

1) The delay in the endogenous IgE production in foals (Wagner et al., 2006b; Marti et al., 2009) could result in less IgE bound to mast cell and basophil surfaces and consequently less severe inflammatory reactions after allergen exposure. However, sensitization of skin mast cells with IgE was observed for almost all horses between 1 and 3 years old and sensitization with IgE in young and adult horses was not different. The skin reactions observed after anti-IgE injection also confirmed that the amount of IgE bound to mast cell surface in young horses was sufficient to mediate degranulation and inflammatory mediator release. Thus, it is unlikely that the suppression of endogenous IgE production during the first 6–9 months of life directly influences the late onset of clinical signs of allergy in horses.

2) The development of an IgE response to Culicoides could simply require time and/or repeated exposure. The response would then gradually increase with age and at some point cross the threshold to develop clinical allergy. This was not supported by our findings, because sensitization to Culicoides did not increase between 1 and 3 years of age. Only one positive skin reaction to Culicoides was observed for the young horses of each of the ages 1, 2 or 3 years. Similarly, an obvious increase in sensitization to Culicoides with age was not observed for the adult horses. We concluded that the production of allergen-specific IgE and sensitization of mast cells with these antibodies alone is not sufficient to explain the development of allergy in horses.

It is not yet fully understood which immune reactions determine whether a horse produces IgE in response to Culicoides exposure and becomes sensitized, and whether or not a sensitized animal develops clinical signs of allergy. Regulatory immune mechanisms might play an important role in the development of clinical allergy in humans. Successful desensitization in human allergy patients was linked to the induction of interleukin 10 (IL-10) and/or tumor growth factor-\(\beta\) (TGF-\(\beta\)) secreting regulatory T-cells (Hawrylowicz and O’Garra, 2005; Akdis and Akdis, 2007; Pereira-Santos et al., 2008; Ryanna et al., 2009). Regulatory T-cells directly suppressed the allergen-specific T-cell development (Meiler et al., 2008) and the activation of mast cells (Gri et al., 2008). In horses, IL-10 and TGF-\(\beta\) down-regulation of IL-4 suggested an suppressive effect on the development of skin hypersensitivity by this mechanism (Hamza et al., 2008).

Another regulatory mechanism influencing the outcome of mast cell and basophil degranulation is provided by FcyRs mediating both activation or inhibition of the cells (Ujike et al., 1999; Tkaczyk et al., 2004; Bruhns et al., 2005). Simultaneous crosslinking of FcεRI and the inhibitory FcγRIIB resulted in decreased activation and degranulation from human basophils (Kepley et al., 2000; Wigginton et al., 2008). In contrast, aggregation of the FcγRI on human mast cells (Woolhiser et al., 2001) or FcγRIII on murine mast cells (Miyajima et al., 1997) promoted...
mediator release comparable to crosslinking of FcεRI. In the horse, FcγRs on mast cells or basophils have not yet been identified. Nevertheless, equine IgG antibodies could play a role in modulating the allergic responses in adult horses and could be even more important in young horses before endogenous IgE is produced.

That anti-IgG(T) mAb CVS40 was previously found to induce immediate skin reactions in horses suggested direct sensitization of mast cells with IgG(T) (Wagner et al., 2006a). Here, we investigated whether IgG(T) might have a role in the sensitization of skin mast cells and basophils early in life. Although the previous skin testing results with the anti-IgG(T) antibody CVS40 were repeatable in this study in adult horses, evidence for an activating role of IgG(T) in the sensitization of skin mast cells or basophils early in life could not be found. The responsiveness of young horses and foals to the CVS40 mAb in skin testing and the in vitro degranulation assay was lower than that for anti-IgE. This confirmed the primary role of IgE in sensitization of equine mast cells and basophils and its potential to mediate the age-independent activation of these cells. The inability of various other anti-IgG3 and/or anti-IgG5 mAbs to induce immediate skin reactions could be interpreted as evidence that skin mast cells are not likely to be directly sensitized with these isotypes and that the skin reactions induced by CVS40 might be mediated by other, IgG-independent or indirect mechanisms. Potentially, CVS40 could have an indirect effect on degranulation by formation of IgG(T)/anti-IgG(T) complexes followed by binding to activating FcγRs in adult horses. In humans, IgG-mediated mast cell degranulation depended on the FcγR and on the mast cell type. Biological responses of mast cells were a result of the balance between positive and negative signals that were generated by various activating and inhibitory receptors on the mast cell surface (Malbec and Daéron, 2007). Mast cells and basophils of young horses might have a different FcγR expression profile resulting in the observed age-dependent variations in degranulation induced by the CVS40 mAb.

Cross-reactions of the anti-IgG(T) mAb CVS40 with IgE have been previously excluded for soluble IgE by serological assays (Wagner et al., 2003, 2006a). However, it cannot be completely ruled out that IgE binding to FcεRI induces conformational changes of the IgE molecule that supported the binding of CVS40 to the receptor-bound IgE and thereby mediated degranulation. If the induction of degranulation by the CVS40 mAb was a result of cross-reactivity with receptor-bound IgE, the decreased degranulation in young horses could be explained by a lower density of FcεRI-bound IgE on the cell surface of mast cells and basophils in young horses that was not sufficient to induce degranulation by CVS40 effectively.

Foals do not produce IgE for the first months of life. However, maternal IgE antibodies are transferred to the foal with the colostrum and can be detected in the circulation for several weeks. In neonates and foals, basophils are thus solely sensitized with maternal IgE (Wagner et al., 2006b). At birth, the total histamine content and the IgE-mediated histamine release from neonatal cells was low or not detectable. Between birth and 12 weeks of life, the total histamine content and the anti-IgE-mediated histamine release increased in the samples from the foals, although the amount of maternal IgE on basophil surfaces decreased during the same time (Wagner et al., 2006b). Our current findings suggested that neonatal basophils had an impaired ability to provoke inflammatory reactions because they contained very low concentrations of histamine, and that both the histamine content and the ability of basophils to release histamine by IgE-mediated mechanisms increased during the first weeks of life. At 12 weeks of age, the total histamine content of foal cells did not differ from cells of adult horses.

Crosslinking of maternal IgE antibodies on neonatal basophils could be induced by environmental antigens after birth and might provide an initial antigen-specific trigger of the foal’s immune response early in life. Such a mechanism might induce an adaptive immune response in the foal that depends on the mare’s acquired immunity. This could be beneficial for the foal if IgE antibodies to parasites are transferred or it could induce a first allergen-specific response if the mare is allergic. Nevertheless, the reduced ability of the neonatal and young foal’s basophils to release histamine might prevent strong inflammatory responses very early in life.

Horses provide a valuable animal model for the better characterization and understanding of the late onset of allergic diseases despite early exposure to allergen and the development of functional IgE and competent mast cell and basophil responses during the first year of life. Investigations of the immune response induced by maternal IgE and the mechanisms that stimulate endogenous IgE production in foals could lead to a novel understanding of the immune regulation during the preclinical development of allergic diseases and the identification of natural resistance mechanisms in genetically predisposed individuals.

5. Conclusion

Sensitization of skin mast cells or basophils in young and adult horses occurs primarily by IgE antibodies. We could not confirm our hypothesis that IgG(T) antibodies have a role in sensitization early in life. Sensitization with maternal IgE was detected during the first 3 months of life. Sensitization with endogenous IgE could be observed as early as 1 year of age. Culicoides extract was the most prevalent allergen in young horses. Until 3 years of age, the sensitization status to Culicoides allergen did not vary with age but it increased in adult horses.

Conflict of interest

None.

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