Veterinary Immunology and Immunopathology 127 (2009) 203–211

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Maternal transfer of IgE and subsequent development of IgE responses in the horse (*Equus callabus*)

Eliane Marti [a], Felix Ehrensperger [c], Dominik Burger [d], Jennifer Ousey [e], Michael J. Day [b], A. Douglas Wilson [b,*]

[a] *Division of Clinical Research, Department of Clinical Veterinary Medicine, Bremgartenstr. 109a, P.O. Box, 3001-Berne, Switzerland*
[b] *Division of Veterinary Pathology, Infection and Immunity, University of Bristol, School of Clinical Veterinary Science, Langford House, Langford BS40 5DU, UK*
[c] *Institute of Veterinary Pathology, Immunopathology Group, Vetsuisse Faculty, University of Zürich, Winterthurerstrasse 268, CH-8057 Zürich, Switzerland*
[d] *Swiss National Stud, 1580 Avenches, Switzerland*
[e] *Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk CB8 7UU, UK*

ARTICLE INFO

Article history:
Received 20 March 2008
Received in revised form 24 September 2008
Accepted 2 October 2008

Keywords:
IgE
Maternal antibody
Mast cell
Allergy
Immune development

ABSTRACT

Immunoglobulin E (IgE) mediates the immune response to parasites, but can also cause allergies. In humans maternal IgE is not transferred to cord blood and high levels of cord blood IgE are associated with subsequent allergy. In horses, both maternal IgG and IgE are transferred via colostrum; the IgE levels in the mare's serum, the colostrum and the foal's serum are correlated but the consequences of IgE transfer to foals are not known. By about 6 weeks of age the levels of IgE in foal serum have dropped to a nadir, at 6 months of age the level of IgE has risen only very slightly and is no longer correlated with the levels seen at birth, IgE^+ B-cells could be detected in lymphoid follicles of some foals at this age. Surprisingly, the levels of total IgE detected in a foals serum at 6 months of age are significantly correlated with the level in its serum at 1, 2 and even 3 years of age suggesting that by 6 months of age the foals are synthesizing IgE and that a pattern of relatively higher or lower total serum IgE has been established. The neonatal intestinal mucosa contained connective tissue mast cells which stained for bound IgE in foals up to 9 weeks of age but not mucosal mast cells, thereafter, the intestinal mast cells were IgE negative until 6 months of age. IgE antibodies to *Culicoides nubeculosus* salivary antigens were detected in Swiss born foals from imported Icelandic mares allergic to *Culicoides spp.* yet the foals showed no signs of skin sensitization and such second generation foals are known not to have an increased risk of developing allergy to *Culicoides*. Overall this evidence suggests there is a minimal effector role of maternal IgE also that maternal IgE has waned prior to the onset of IgE synthesis in foals and does not support maternal priming of IgE responses in foals. Furthermore the total levels of IgE in any given foal are seen to be relatively high or low from soon after the onset of IgE synthesis, and most likely they are determined by genetic factors.

1. Introduction

Although immunoglobulin E (IgE) is the least abundant serum immunoglobulin, it is arguably the most potent as a mediator of acute inflammatory reactions to foreign antigens. IgE antibody responses have a central role both in allergies and in protection against helminth parasites (Reddy and Fried, 2008). In horses, IgE has been shown to be important in the pathogenesis of allergy to insect bites (Hellberg et al., 2006; Langner et al., 2008) and to a lesser extent may play a role in recurrent airway obstruction (Kunzle et al., 2007). Both IgE and IgG appeared early in

* Corresponding author. Tel.: +44 117 331 9067.
E-mail address: doug.wilson@bris.ac.uk (A.D. Wilson).

0165-2427/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2008.10.316

mammalian evolution and are also present in marsupials and monotremes such as the duck-billed platypus (Vernersson et al., 2002). IgE heavy chain contains four immunoglobulin domains compared to three domains of IgG, and this structure is thought to have first evolved by gene duplication from ancestral four domain immunoglobulin IgY found in reptiles and birds with the subsequent loss of one immunoglobulin domain by IgG (Warr et al., 1995). Through time the number and function of IgG molecules has diversified in different species with marsupials and monotremes typically having two IgG isotypes (Vernersson et al., 2002), while humans, rats and mice have four IgG genes (Bruggemann, 1988; Flanagan and Rabbitts, 1982; Knight et al., 1985; Shimizu et al., 1982), swine have five IgG isotypes (Butler and Brown, 1994), horses have seven (Wagner et al., 2004) cattle have three (Zhao et al., 2003) and rabbits only have one (Knight et al., 1985). In contrast, the structure and single copy number of the IgE gene has remained conserved throughout evolution in all of the species studied so far (Clarke and Beh, 1993; Knight and Becker, 1987; Navarro et al., 1995; Vernersson et al., 2004; Zhao et al., 2003).

The potency of IgE is due to its ability to bind high affinity Fcε receptors on the surface of mast cells and basophils then mediate the release of histamine, prostaglandins, leukotrienes, proteases and several cytokines in response to antigen binding (Gould et al., 2003; Kawakami and Galli, 2002). These cytokines and pro-inflammatory products can promote B-cells IgE synthesis (Pawankar et al., 1997) and enhance T-helper 2 responses either directly or through altering dendritic cell function (Caron et al., 2001; Mekori and Metcalfe, 1999). Also IgE bound to Fcε on monocytes and dendritic cells enables them to efficiently take up and present low concentrations of soluble antigens including excretory/secretory antigens produced by helminth parasites (Baeza et al., 2004; Bakker et al., 2004; Getahun and Heyman, 2004). Once an IgE response is established, the combined effects of enhanced antigen presentation and release of mast cell cytokines form a positive feed back mechanism which maintains the response. Maintenance of an effective anti-parasite response may underlie the co-evolution of the conserved IgE and mast cell interaction but this also carries the risk of developing allergic disease which has a high and increasing incidence in affluent societies. One reason put forward to explain this is the ''hygiene hypothesis'' which proposes a lack of exposure to bacterial antigens, that would otherwise lead to a more T-helper1 dominated response, combined with low levels of parasite specific IgE, reduce the threshold at which IgE responses to environmental antigens can sensitize mast cells (Smits et al., 2005).

Another conserved feature of the immune system is the passive transfer of antibodies from mothers to their offspring (Grindstaff et al., 2003). Maternal antibody transfer occurs through the yolk in the eggs of birds, reptiles and fish (Hassl, 2005; Swain et al., 2006; West et al., 2004), while in mammals antibodies are transferred either across the placenta prenatally or are secreted in colostrum and absorbed intact from the neonate's intestine (Grindstaff et al., 2003). For human infants the transfer of immunoglobulin to cord blood is limited to IgG which crosses the placenta by neonatal Fc receptor specific transcytosis (Avrech et al., 1994; Leach et al., 1996). In contrast, for ruminants and horses both maternal IgG (Mayer et al., 2002, 2005; Wilson et al., 2001) and maternal IgE (Pfeffer et al., 2005; Thatcher and Gershwin, 1989; Wagner et al., 2006) are secreted at high concentrations into the colostrum then absorbed intact from the intestine during the first 24 h of life. The function of maternal IgE transfer and its effects on the developing neonatal immune system and why maternal transfer of IgE only occurs in some species is not known. To further address this issue, in the present study we have examined some aspects of mast cell development and IgE immune responses in foals.

2. Materials and methods

2.1. Subjects and blood sampling

Blood and colostrum samples were collected at foaling from a cohort of 40 Swiss warmblood mares at the Swiss national stud during the 1993 and 1994 foaling seasons. Subsequently, the foals were bled at 6 months, then at 1, 2 and 3 years of age. Foals born the same year were kept together in a group on the stud farm during the whole sampling period, they grazed the same pastures and received the same supplementary hay and feed. The foals were de-wormed on a regular basis (3–4× per year) and were vaccinated against influenza and tetanus. Blood samples from a separate group of 16 UK foals were collected at 6 weeks of age, at 5 months of age (prior to weaning) and then post-weaning at 9 months of age. This group were part of a separate study on diet and development, the details of which have been described elsewhere (Wilson et al., 2007). Samples of pre-suck serum were collected from six foals at the Equine Fertility Unit, Newmarket. All samples were allowed to clot and separated serum was stored at −20 °C until assayed.

2.2. Total IgE ELISA

Total serum IgE concentration was measured by antibody capture ELISA using our own monoclonal antibodies (Wilson et al., 2006). Briefly, 96-well polystyrene plates were coated overnight with monoclonal anti-equine IgE (1C12). Plates were washed in PBS–0.05% tween and blocked by incubation for 1 h with 1% BSA in PBS–tween. Each plate had a duplicate standard curve of doubling-dilutions of purified equine IgE from 100 to 3.125 ng/ml, duplicate wells containing no IgE and conjugate blanks. Four tripling dilutions of each sample from 1:30 to 1:810 were placed into test wells, the plates were incubated at room temperature for 2 h, washed and a second biotinylated anti-IgE monoclonal antibody (3H10) was added. Following a 1-h incubation and wash, the plates were incubated with Extravidin alkaline phosphatase conjugate (www.sigmaaldrich.com). After a final wash, colour was developed with *p*-nitrophenyl phosphate substrate and the plates were read by spectrophotometer. The data were analysed using Genesis software (Labsystems Multiskan EX). The IgE

concentration was calculated from an average of the test sample dilutions that fell within the standard curve.

2.3. Immunohistochemistry

Blocks of formalin-fixed intestinal tissue from a total of 25 foals of varying ages were retrieved from the archives of the Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zürich, and the Division of Veterinary Pathology, Infection and Immunity, School of Clinical Veterinary Science, University of Bristol. H and E stained sections were examined those in which the tissues appeared autolysed or unhealthy were excluded, replicate sections were stained with toluidine blue to detect tissue mast cells (du Toit et al., 2007). For immunohistochemical detection of IgE, the sections were de-waxed in Histoclear (www.rlamb.net), re-hydrated through graded alcohols and water. Dual Endogenous Enzyme block (DAKO) was applied to inactivate tissue and intestinal alkaline phosphatase. The sections were then incubated with 1 mg/ml trypsin-250 (Difco) in 1 mg/ml calcium chloride buffer pH 7.8 for 30 min at 37 °C. Slides were then washed and blocked with PBS–2%BSA and incubated with monoclonal anti-horse IgE clone number 7.A2 (Wilson et al., 2006) for 1 h followed by donkey anti-mouse biotin conjugate (www.jacksonimmuno.com) and streptavidin-alkaline phosphatase complexes (www.dakocytomation.com). Colour was developed with Fast Red RC, NaptholAS-MX substrate. Sections were counterstained with 10% Mayer's haematoxylin. Images were captured using a Leica DMLS microscope with a DC300 camera and IM50 image management software.

2.4. Immunoblotting

Separate samples of serum and colostrum were collected from three mares with insect bite hypersensitivity (IBH) together with a sample of foal serum. Allergen-specific IgE was detected in Western blots of salivary protein extracts from *Culicoides spp.* as previously described (Hellberg et al., 2006). Briefly, *Culicoides* salivary proteins were separated in SDS page gels and transferred to PVDF membranes. The membranes were incubated overnight with a 1:6 dilution of serum or colostrum, bound IgE was detected using monoclonal anti-equine IgE (1C12) and a horse radish peroxidase conjugated goat anti-mouse–IgG antibody (Sigma). The blots were developed with ECL Western blotting detection reagent (www.gelifesciences.com).

2.5. Statistical analysis

Data was analysed using SPSS statistical package. The IgE data were not normally distributed therefore they were log transformed prior to analysis. Mean IgE level in mare serum and colostrum were compared using a paired *t*-test.

The serum IgE levels in the first group of 40 Swiss foals was compared using a repeated measures analysis of variance. Between group differences were assessed using the least significant difference. The second group of only 16 foals was analysed using the nonparametric test, Friedman's two-way ANOVA.

Associations between variables were analysed by multiple correlation using Pearson product–moment correlation coefficient r for each pair of serum (or colostrum) values, the combination of all the possible comparisons used to generate a correlation matrix.

3. Results

3.1. Total IgE ELISA

The mean IgE concentration in colostrum samples from 40 mares was 76,065 ng/ml with a standard deviation of ± 56,172 ng/ml and was significantly higher than that of mare serum (mean ± S.D., 43,008 ± 28,502 ng/ml, $p = 0.0002$, paired *t*-test). None of the serum samples taken from six foals prior to sucking had an IgE concentration above the level of the lowest detectable standard of 3 ng/ml (data not shown). In comparison at 24 h post-partum the foals from the 40 mares had absorbed substantial amounts of IgE (mean ± S.D., 10,853 ± 8071 ng/ml).

The total IgE concentration in the mares serum and colostrum were significantly correlated $r = 0.334$ $P = 0.034$. Similarly total IgE concentration in the foals' serum was highly significantly correlated with that found in the either the mare's serum $r = 0.414$ $P = 0.0083$ or colostrum $r = 0.486$ $P = 0.0015$ (Table 1). However, there was no correlation between the level of IgE in foal serum at 24 h of age and that found at 6 months of age $r = 0.128$ $P > 0.05$ indicating that any contribution of maternal IgE to the total serum IgE had waned by this age (Table 1). In contrast, the concentration of IgE in foal serum at 6 months of age was highly significantly correlated with that at 1-year old

Table 1
Correlation matrix of total IgE in mares and foals.

	Colostrum	Mare serum	Foal serum	Six months	One year	Two years	Three years
Colostrum	1.000	**0.335**	**0.486**	−.023	−.229	−.136	−.131
Mare serum	$P = .0340$	1.000	**0.414**	.012	−.091	−.073	−.029
Foal serum	$P = .0015$	$P = .0083$	1.000	.128	−.121	−.097	−.070
Six months	NS	NS	NS	1.000	**0.574**	**0.540**	**0.374**
One year	NS	NS	NS	$P < .0001$	1.000	**0.709**	**0.531**
Two years	NS	NS	NS	$P = .0003$	$P < .0001$	1.000	**0.723**
Three years	NS	NS	NS	$P = .0202$	$P = 0.005$	$P < .0001$	1.000

Table showing the results of a correlation matrix between total IgE in colostrum, mare's serum, foal's serum and the serum samples taken from the foals at different ages. Values for r are plotted on the top right half of the table, those that are significantly different from 0 are in bold. The significance values (P) are plotted in the lower left. NS: not significant.

($r = 0.574$; $P < 0.0001$), at 2 years old ($r = 0.540$; $P = 0.0003$) and significantly correlated with the total serum IgE at 3 years old ($r = 0.374$; $P = 0.0202$). These observations are consistent with the levels at 6 months of age being due to newly synthesised IgE, not residual IgE transferred from the mother.

The data from the foals at 6 months, 1, 2 and 3 years of age was further analysed by repeated measures analysis of variance. There were highly significant differences between the mean total IgE at these time points, total IgE also showed a highly significant linear relationship with time. Multiple pairwise comparisons were made using the least significant difference. Total IgE at 1 year of age was significantly higher than at 6 months of age, similarly the mean serum IgE was significantly higher at 2 years compared to 1 year ($P < 0.01$) and at 3 years compared to 2 years ($P < 0.01$) by which time it had reached a mean of 12,802 ± S.D. 8371 ng/ml but remained significantly less than the adult level found in the mares (mean ± S.D., 76,065 ± 56,172 ng/ml; $P = 0.001$) (Fig. 1).

To examine changes in IgE concentration during the first year of life in more detail, IgE was assayed in a separate group of foals sampled at 6 weeks of age, 6 months of age and 9 months of age (Fig. 2). We were unable to sample these foals at birth to confirm IgE transfer but all the foals were known to have sucked in the first 24 h of life. Assuming that the foals started with serum IgE levels comparable to those in the other group, by 6 weeks of age the level of IgE in most of these foals had fallen to below 500 ng/ml. IgE levels rose in 10 of these foals by 6 months of age but fell in the remaining six so there was no overall significant change in mean total IgE between these time points. Total serum IgE in 13 of the 16 foals rose between 6 and 9 months of age, contributing to a significant overall increase to an average of 318 ng/ml (Friedman's two-way analysis of variance by rank $P = 0.047$) indicating that synthesis of IgE was occurring in foals between 6 and 9 months of age (Fig. 2).

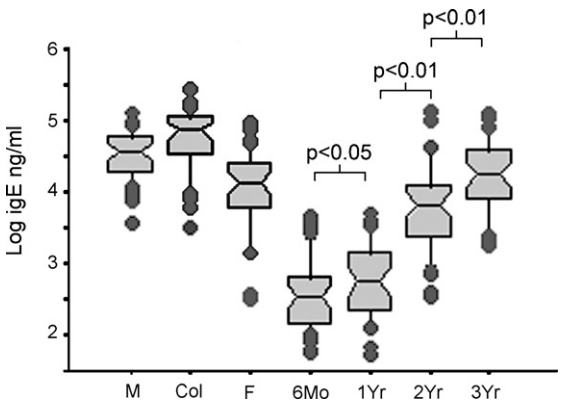

Fig. 1. Box plot of log total IgE in mare serum (M), colostrum (Col), day old foal serum (F), and foal serum at 6 months, 1 year, 2 years and 3 years of age. Box = 25–75th percentile, waist = median ± 95% CI, bars = 10–90th percentile. Repeated measures analysis of variance showed statistically significant differences between the values of total serum IgE in the foals at 6 months 1, 2 and 3 years of age as indicated on the graph.

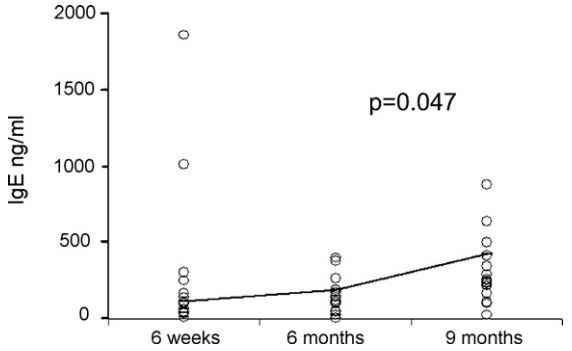

Fig. 2. Dot plot showing the total IgE concentration in foals' serum at 6 weeks, 6 months and 9 months of age. Line equals median value. Friedman's two-way analysis of variance confirmed there were significant differences between the groups $P = 0.047$.

3.2. Immunohistochemistry

To examine the extent to which neonatally transferred IgE may be bound by mast cells we examined sections of intestinal tissue by immunohistochemistry. Connective tissue mast cells were detected in the intestinal sub-mucosa of neonatal foals by toluidine blue staining (Fig. 3a). Similar numbers of IgE^+ cells were detected in the sub-mucosal tissue of 24 h old foals that had received colostrum (Fig. 3b), no sub-epithelial IgE positive cells indicative of the presence of mucosal mast cells were detected in the villi of foals at this age (Fig. 3c). Very strong and extensive labelling was seen in the centre of nascent lymphoid follicles in the intestine of one neonatal foal (Fig. 3d) this was not observed in any other foals of 2 and 3 days of age and may represent a transient localisation of the absorbed IgE in lymphoid cells. At 2 weeks of age, IgE^+ connective tissue mast cells were still detected (Fig. 3e) but again no mucosal mast cells were found (not shown). By 9 weeks of age the intensity of labelling had reduced (Fig. 3f) and by 22 weeks of age the mast cells in the lamina propria were no longer IgE^+ even though they could still be detected by toluidine blue staining (data not shown).

In marked contrast, at 6 months of age we found strong IgE labelling of lymphoid follicles in the intestine and of the mesenteric lymph node (Fig. 3g), providing evidence of active B-cell synthesis of IgE. This was associated with the presence of numerous IgE^+ connective tissue mast cells in the sub-mucosa (Fig. 3h) and IgE^+ cells lying below the villus epithelium consistent with the expected location of mucosal mast cells (Fig. 3i). By 9 months of age the intestine had the same pattern of extensive mucosal and connective tissue mast cell populations seen in sections from adult intestine (Fig. 3j and k).

3.3. Immunoblotting

To determine whether transfer of antigen specific IgE had occurred, three sets of matched mare serum, colostrum and foal serum were collected from families where the mare showed symptoms of hypersensitivity to the bites of *Culicoides spp.* IgE antibodies specific for the salivary proteins of *Culicoides spp.* were detected in the

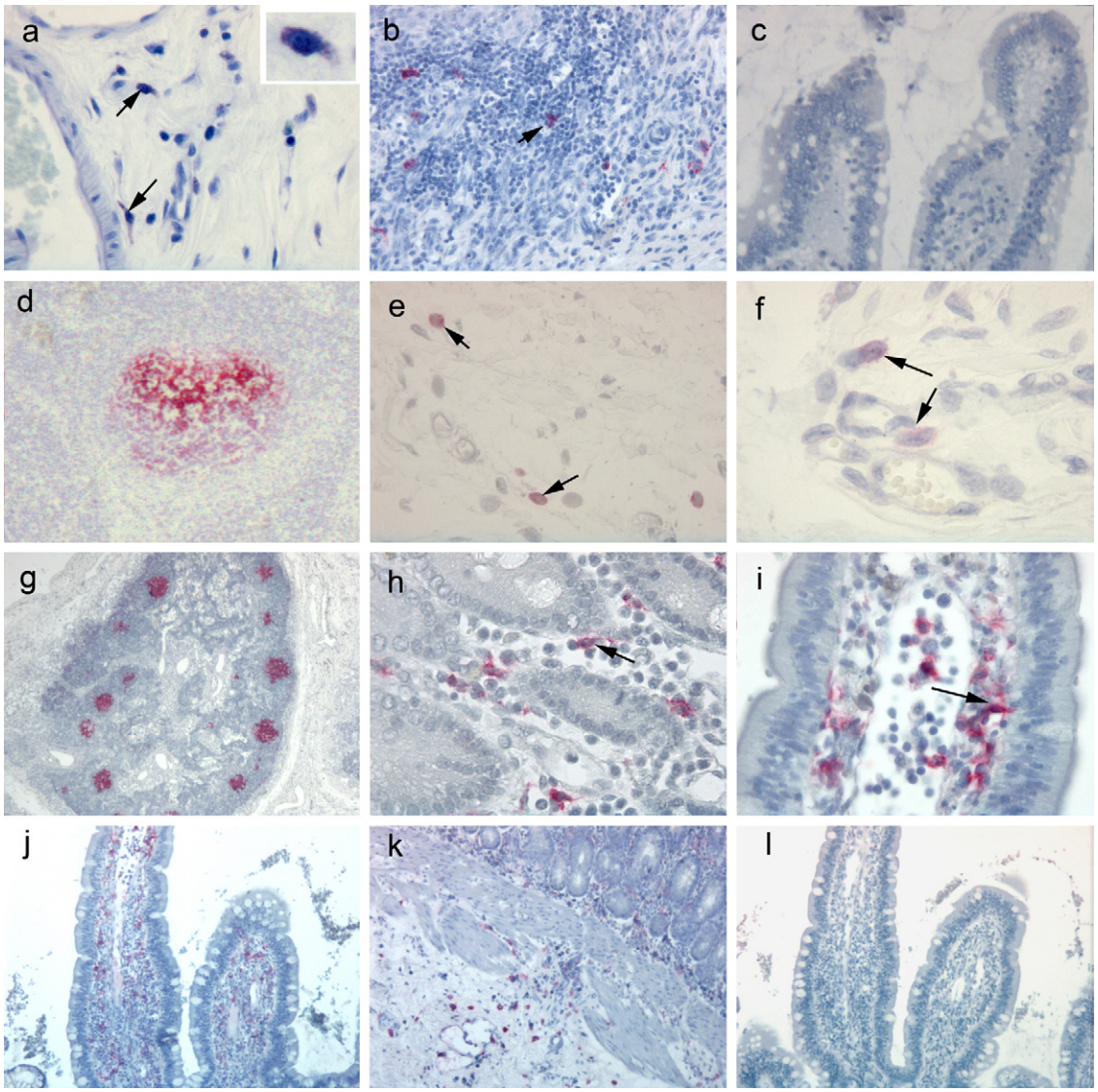

Fig. 3. (a) Sub-mucosal connective tissue mast cells (arrows) in neonatal foals stained with toluidine blue (40×) inset shows enlargement of mast cell. (b) IgE positive cells (arrow) in the sub-mucosa of colostrum fed 24 h old foal (20×). (c) Absence of IgE labelling in the intestinal villi of a 24-h old colostrum fed foal (20×). (d) IgE accumulation within a developing intestinal lymphoid follicle of a colostrum fed foal at 24 h of age (10×). (e) and (f) Reduced intensity of IgE labelling of connective tissue mast cells (arrows) in foals between 2 and 9 weeks of age (40×). (g) Mesenteric lymph node in a 6-month old foal showing IgE positive follicles in the medulla indicative of de-novo IgE synthesis (10×). (h) IgE positive mast cells in the lamina-propria of a 6-month-old foal (40×). (i) IgE positive mucosal mast cells (arrow) in the intestinal villi of a 6-month-old foal (40×). (j) Intestinal villi of adult stained for IgE (10×). (k) Lamina propria and sub-mucosal IgE positive cells adult intestine (10×). (l) Negative control staining of adult intestinal villi with isotype control antibody (10×).

mare serum, in colostrum and in the serum of the foals from all three allergic groups. Unexpectedly we also detected IgE antibodies to *Culicoides* salivary proteins in the colostrum from normal non-sensitised mares although there were only faint traces of IgE antibody found in the serum of either of the normal mares or their foals (Fig. 4).

4. Discussion

The serum samples in this study were stored for several years at −20 °C before being assayed. IgE has a reputation for instability compared to other immunoglobulin isotypes (Lehrer, 1977) based on the observation that the passive sensitisation of mast cells by intradermal injection of IgE is abolished by heating to 56 °C. This instability relates to the biological activity of IgE which is dependant on its binding the Fcε receptor on mast cells. The monoclonal antibodies used in our assay measures the presence of two epitopes on the IgE molecule; we have shown that these epitopes are not abolished by heating to 56 °C which had no effect on the titre of IgE antibodies in our ELISA (unpublished observation). Further evidence of the stability of IgE in storage comes from out comparison of the original IgE standard used in our ELISA (prepared several years ago and stored at −20 °C) with a freshly prepared standard which shows an identical titre of IgE in the ELISA.

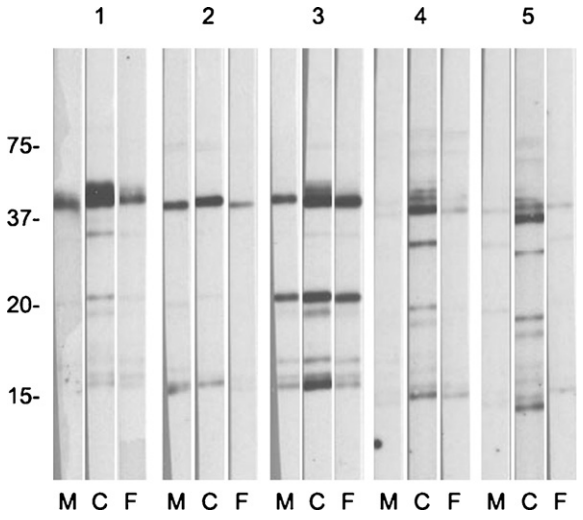

Fig. 4. IgE antibodies reacting with Western blot of salivary proteins from *Culicoides nubeculosus*. M: mare serum, C: colostrum, F: foal serum. Samples 1–3 were taken from mares with clinical allergy to *Culicoides spp.*, and show clear transfer of IgE antibodies to *Culicoides* proteins from mother to foal. Samples 4 and 5 were from healthy mares, IgE antibodies to *Culicoides* proteins are barely detectable in the serum of both mares and foals. Surprisingly IgE antibodies to *Culicoides* were also detectable in the colostrum of the healthy mares which may reflect the higher concentrations of IgE found in mares colostrum.

Transfer of maternal IgG or IgY to offspring is a universal characteristic of the vertebrate immune system. This is an important adaptive immunological mechanism and failure of adequate transfer is associated with high neonatal mortality in many species including horses (Grindstaff et al., 2003; McGuire et al., 1977). Placental transfer of IgG in humans and absorption of IgG from the neonatal intestine of rats occurs by Fc receptor-mediated transcytosis and is limited to some IgG isotypes (Avrech et al., 1994; Leach et al., 1996; Yoshida et al., 2006). In ruminants, and pigs macromolecules are taken up from the neonatal intestine by a route that has characteristics of both IgG receptor specific mechanisms and non-specific endocytosis of macromolecules (Staley and Bush, 1985; Westrom et al., 1985). In horses maternal transfer is also via colostrum and most IgG isotypes are known to be transferred (Wilson et al., 2001) with IgG2b having the highest concentration in neonates (Holznagel et al., 2003). The reported half-life of transferred IgG in foals was between 4 and 5 weeks depending on the isotype where again IgG2b showed the slowest rate of decline (Holznagel et al., 2003). Maternal IgG was still detectable in foals of 6 months of age (Wilson et al., 2001) by comparison IgE in foals had all but disappeared by 6 weeks (Fig. 2) suggesting that it has a shorter half life. These figures for foals up to 6 weeks of age agree with the results reported in a similar study (Wagner et al., 2006), in which serum IgE was found to be below the limit of detection between 4 and 9 months old. In contrast our results for total serum IgE in either the foals between 6 months and 1 year of age (Fig. 1) or between 6 weeks and 9 months of age (Fig. 2) indicated that there was IgE synthesis by 6 months of age, the results of immunohistology confirmed IgE positive B-cell follicles in the lymph nodes of a 6-month-old foal. Furthermore, although there was no correlation between total serum IgE in neonates and 6-month-old foals, the levels of total IgE foals at 6 months were correlated with those in the same individual at 1, 2 and even 3 years of age. These observations support the conclusion that synthesis of IgE has commenced at 6 months of age, by which time it is established whether foals will have relatively high or low IgE as adults. As these foals were all maintained in groups throughout the study, this observation together with the lack of any correlation between maternally transferred IgE and total IgE in at later ages indicates the possibility of genetic influences on IgE synthesis in horses as has been reported for humans (Kabesch et al., 2006; Pinto et al., 2007).

Maternal IgE is only transferred in some species such as horses and ruminants, raising the questions of how this molecule is transferred and whether this transfer has functional significance. The mechanism by which IgE is absorbed from the neonatal foal's intestine is not known. It is possible that IgE is absorbed as a bystander antigen associated with the enhanced endocytosis triggered by IgG in the colostrum (Westrom et al., 1985). Alternatively, CD23-mediated transcytosis of IgE complexes is known to occur in the intestine of rodents and humans (Li et al., 2006; Yu et al., 2001) and this would provide a potential specific mechanism for absorption of IgE from the intestine of neonatal foals and ruminants. It is also unclear how the IgE first moves from the maternal blood into the colostrum. The central consideration is whether or not IgE is actively transported and therefore significant, or whether IgE has simply arrived by a passive mechanism such as diffusion through the epithelial lining of the mammary gland and non-specific endocytosis in the intestine; in which case its presence may be coincidental and of no biological importance. The higher concentration of both IgG and IgE in equine colostrum compared to serum, suggests that these molecules may be actively transported. In cows and sheep there is a selective bias for IgG1 transport into colostrum by FcRn receptors in the mammary gland epithelium (Mayer et al., 2005; Mayer et al., 2002), and FcRn has also been identified in the mammary gland of pigs at parturition (Schnulle and Hurley, 2003). If as the high levels of colostral IgE suggest, a similar active mechanism has evolved to transport IgE in horses and ruminants this would imply that it confers an advantage on their offspring.

In horses the most obvious advantage of maternal IgE transferred to foals would be protection from helminth parasites such as *Strongyloides westeri* which is transferred to suckling foals in milk and *Parascaris equorum* whose sticky eggs are also readily picked up by suckling foals (Russell, 1948). IgE positive mast cells were detected in the intestine up to 9 weeks of age which is longer than IgE positive circulating leukocytes (basophils) that were reported to be strongly IgE positive at 5 days of age after which the numbers dropped rapidly to become undetectable by 6 weeks (Wagner et al., 2006). However, in the foal intestine, mast cells were limited to connective tissue types present in low numbers with no mucosal mast cells detected until 6 months of age, raising doubts as to

whether the neonatal intestine has a fully effective an anti-parasitic response. Maternal IgE could arguably provide an advantage to the offspring by stimulating the maturation and development of mast cells (Kawakami and Galli, 2002) but this goes against the prevailing evidence showing only transient IgE binding by mast cells with no clear increase in either numbers or intensity of IgE staining cells in the 6 weeks after birth. Mast cells of the intestine can be divided into connective tissue and mucosal populations based on location and differences in their granule content. Toluidine blue is used in all species including horses as a mast cell stain for connective tissue mast cells but does not stain mucosal mast cells which are defined by differences in their sulphated glycosaminoglycan granule content and proteolytic enzyme activities (du Toit et al., 2007). Mast cell numbers and their differentiation into subtypes is governed by the local cytokine microenvironment (Kawakami and Galli, 2002) and the absence of IgE positive cells in the intestinal villi of foals less than 6 months of age may reflect the overall immaturity of the mucosal immune system at this age.

It was not possible to test for the transfer of parasite specific IgE but antigen-specific IgE transfer was detected in the serum of foals born to mares with allergy to *Culicoides spp*. Such foals show no clinical evidence of skin irritation even though they are exposed to insect bites in the first few weeks of life, again raising doubts as to whether maternal IgE can efficiently sensitise mast cells in foals. Further studies are required in order to determine whether this lack of response reflects an absence of mast cells in the skin of foals, or a failure of IgE sensitization of these cells which may occur if the wide range of antigen specificities in equine IgE results in too low a density of antibodies for any one specificity that may be required to efficiently trigger mast cell de-granulation.

Other indirect benefits of maternal IgE transfer would be conferred if these molecules were able to prime an anti-parasite immune response. This could be mediated by the release of cytokines such as IL-4 from mast cells in response to antigen challenge or through IgE bound to antigen presenting cells, thus increasing their efficiency of soluble antigen uptake. Although peripheral blood monocytes from adult horses have been shown to express Fcε (Wagner et al., 2003) their presence has not been confirmed in foals. A high concentration of IgE in the centre of nascent lymphoid follicles of the intestine was found in at 24 h of age but this rapidly disappeared and was not detected in older foals (Fig. 3d). There is considerable debate in human medicine as to the relationship between high cord blood IgE concentration and the subsequent development of clinical allergy and some studies have pointed out that an allergic mother provides a higher risk than an allergic father, suggesting that environmental/maternal factors also play a role in priming allergic responses (Muraro et al., 2004). The detection in human cord blood of immune complexes containing allergen and IgE may indicate de-novo IgE synthesis but could also be explained by trans-placental transfer. Maternal antibody in humans is restricted to IgG isotypes transferred across the placenta by FcRn mediated transcytosis (Avrech et al., 1994; Leach et al., 1996), but it is not possible to rule out inadvertent movement of both allergen and IgE in trace amounts complexed to IgG (Bertino et al., 2006).

Other studies have linked high levels of cord blood IgE to genetic factors such as polymorphisms in the IL-4 receptor and concluded that it is due to increased de-novo synthesis of IgE by the foetus (Wen et al., 2006). The data in the present study shows no relationship between the total IgE transferred to foals at birth and IgE levels at later ages. The correlation between the IgE levels at 6 months of age with those found at 1, 2 and 3 years of age supports a similar genetic determination of high and low IgE responders occurring in horses, and not a reflection of high maternal IgE leading to higher IgE synthesis in foal.

Allergy to Culicoides is one of the commonest skin diseases in horses and we provide clear evidence that maternal IgE specific for *Culicoides* salivary antigens is transferred to newborn foals. However, the epidemiology of *Culicoides* hypersensitivity in Icelandic horses provides strong evidence against maternal priming of allergy in horses. There are no *Culicoides* insects in Iceland where allergy to insect bites is not reported, but native Icelandic ponies exported to mainland Europe and first exposed to *Culicoides* as adults have an incidence of allergy to *Culicoides* of around 26%. In comparison, the offspring of these horses born on mainland Europe will receive maternal IgE to *Culicoides* antigens and be exposed to *Culicoides* from birth, yet have an incidence of 8.2% which is near to that of the indigenous horse population (Brostrom et al., 1987; Halldordsottir and Larsen, 1991).

Human allergic reactions vary in severity from an inconvenient and unpleasant dermatitis to acutely fatal anaphylactic reactions and over the last two decades allergies have become increasingly common in wealthy modern societies. One proposed explanation for this increase has been the ‘‘hygiene hypothesis. Nevertheless, allergic diseases are not uncommon in domestic animals, even though they have invariably been exposed to the abundant bacterial and parasite antigens in their environment. Given the highly conserved nature of the IgE/mast cell system throughout evolution it may make sense to view allergy as a result of over-dominance, where the advantages of a trait, in this case immunity to parasites, outweigh the disadvantages of potential allergic disease. The difficulty in maintaining this balance may explain why the maternal IgE transferred in horses disappears before the onset of IgE synthesis at around 6 months of age, when foals are becoming increasingly exposed to parasite larvae present on pasture, and why delayed exposure to biting insects such as *Culicoides* is a major risk factor for allergy in Icelandic ponies imported into mainland Europe.

5. Conclusion

Although there is clear evidence for the transfer of maternal IgE in horses, the functional significance remains unclear. The mast cell populations in the intestine of foals are immature and do not develop an adult phenotype with IgE positive mucosal and connective tissue mast cells until endogenous IgE synthesis is established. Evidence from insect bite hypersensitivity does not support the maternal

priming of IgE responses despite clear evidence the allergen specific maternal IgE is transferred.

Acknowledgement

This work was supported by the Swiss National Science Foundation Grant No. 31-63449.00.

References

Avrech, O.M., Samra, Z., Lazarovich, Z., Caspi, E., Jacobovich, A., Sompolinsky, D., 1994. Efficacy of the placental barrier for immunoglobulins: correlations between maternal, paternal and fetal immunoglobulin levels. Int. Arch. Allergy Immunol. 103, 160–165.

Baeza, M.L., Rodriguez, A., Matheu, V., Rubio, M., Tornero, P., de Barrio, M., Herrero, T., Santaolalla, M., Zubeldia, J.M., 2004. Characterization of allergens secreted by Anisakis simplex parasite: clinical relevance in comparison with somatic allergens. Clin. Exp. Allergy 34, 296–302.

Bakker, N., Vervelde, L., Kanobana, K., Knox, D.P., Cornelissen, A.W., de Vries, E., Yatsuda, A.P., 2004. Vaccination against the nematode *Haemonchus contortus* with a thiol-binding fraction from the excretory/secretory products (ES). Vaccine 22, 618–628.

Bertino, E., Bisson, C., Martano, C., Coscia, A., Fabris, C., Monti, G., Testa, T., Conti, A., 2006. Relationship between maternal- and fetal-specific IgE. Pediatr. Allergy Immunol. 17, 484–488.

Brostrom, H., Larsson, A., Troedsson, M., 1987. Allergic dermatitis (sweet itch) of Icelandic horses in Sweden: an epidemiological study. Equine Vet. J. 19, 229–236.

Bruggemann, M., 1988. Evolution of the rat immunoglobulin gamma heavy-chain gene family. Gene 74, 473–482.

Butler, J.E., Brown, W.R., 1994. The immunoglobulins and immunoglobulin genes of swine. Vet. Immunol. Immunopathol. 43, 5–12.

Caron, G., Delneste, Y., Roelandts, E., Duez, C., Bonnefoy, J.Y., Pestel, J., Jeannin, P., 2001. Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. J. Immunol. 167, 3682–3686.

Clarke, R.A., Beh, K.J., 1993. Organisation of the ovine immunoglobulin C epsilon gene locus: evidence for a deletion 5′ of the gene. Vet. Immunol. Immunopathol. 39, 381–393.

du Toit, N., McGorum, B.C., Pemberton, A.D., Brown, J., Dacre, K.J., 2007. The involvement of mast cells and mast cell proteinases in the intestinal response to equine cyathostomin infection. Vet. Immunol. Immunopathol. 115, 35–42.

Flanagan, J.G., Rabbitts, T.H., 1982. Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing gamma, epsilon and alpha genes. Nature 300, 709–713.

Getahun, A., Heyman, B., 2004. IgG- and IgE-mediated antigen presentation on MHC class II. Immunol. Lett. 92, 33–38.

Gould, H.J., Sutton, B.J., Beavil, A.J., Beavil, R.L., McCloskey, N., Coker, H.A., Fear, D., Smurthwaite, L., 2003. The biology of IGE and the basis of allergic disease. Annu. Rev. Immunol. 21, 579–628.

Grindstaff, J.L., Brodie 3rd, E.D., Ketterson, E.D., 2003. Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. Proc. Biol. Sci. 270, 2309–2319.

Halldordsottir, S., Larsen, H.J., 1991. An epidemiological study of summer eczema in Icelandic horses in Norway. Equine Vet. J. 23, 296–299.

Hassl, A., 2005. Snake egg immunoglobulins: biochemical characteristics and adjusted isolation procedure. J. Immunol. Methods 297, 253–257.

Hellberg, W., Wilson, A.D., Mellor, P., Doherr, M.G., Torsteinsdottir, S., Zurbriggen, A., Jungi, T., 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.

Holznagel, D.L., Hussey, S., Mihalyi, J.E., Wilson, W.D., Lunn, D.P., 2003. Onset of immunoglobulin production in foals. Equine Vet. J. 35, 620–622.

Kabesch, M., Schedel, M., Carr, D., Woitsch, B., Fritzsch, C., Weiland, S.K., von Mutius, E., 2006. IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. J. Allergy Clin. Immunol. 117, 269–274.

Kawakami, T., Galli, S.J., 2002. Regulation of mast-cell and basophil function and survival by IgE. Nat. Rev. Immunol. 2, 773–786.

Knight, K.L., Becker, R.S., 1987. Isolation of genes encoding bovine IgM, IgG, IgA and IgE chains. Vet. Immunol. Immunopathol. 17, 17–24.

Knight, K.L., Burnett, R.C., McNicholas, J.M., 1985. Organization and polymorphism of rabbit immunoglobulin heavy chain genes. J. Immunol. 134, 1245–1250.

Kunzle, F., Gerber, V., Van Der Haegen, A., Wampfler, B., Straub, R., 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.

Langner, K.F., Darpel, K.E., Drolet, B.S., Fischer, A., Hampel, S., Heselhaus, J.E., Mellor, P.S., Mertens, P.P., Leibold, W., 2008. Comparison of cellular and humoral immunoassays for the assessment of summer eczema in horses. Vet. Immunol. Immunopathol. 122, 126–137.

Leach, J.L., Sedmak, D.D., Osborne, J.M., Rahill, B., Lairmore, M.D., Anderson, C.L., 1996. Isolation from human placenta of the IgG transporter, FcRn, and localization to the syncytiotrophoblast: implications for maternal-fetal antibody transport. J. Immunol. 157, 3317–3322.

Lehrer, S.B., 1977. Role of mouse IgG and IgE homocytotropic antibodies in passive cutaneous anaphylaxis. Immunology 32, 507–511.

Li, H., Nowak-Wegrzyn, A., Charlop-Powers, Z., Shreffler, W., Chehade, M., Thomas, S., Roda, G., Dahan, S., Sperber, K., Berin, M.C., 2006. Transcytosis of IgE-antigen complexes by CD23a in human intestinal epithelial cells and its role in food allergy. Gastroenterology 131, 47–58.

Mayer, B., Doleschall, M., Bender, B., Bartyik, J., Bosze, Z., Frenyo, L.V., Kacskovics, I., 2005. Expression of the neonatal Fc receptor (FcRn) in the bovine mammary gland. J. Dairy Res. 72, 107–112 special issue.

Mayer, B., Zolnai, A., Frenyo, L.V., Jancsik, V., Szentirmay, Z., Hammarstrom, L., Kacskovics, I., 2002. Redistribution of the sheep neonatal Fc receptor in the mammary gland around the time of parturition in ewes and its localization in the small intestine of neonatal lambs. Immunology 107, 288–296.

McGuire, T.C., Crawford, T.B., Hallowell, A.L., Macomber, L.E., 1977. Failure of colostral immunoglobulin transfer as an explanation for most infections and deaths of neonatal foals. J. Am. Vet. Med. Assoc. 170, 1302–1304.

Mekori, Y.A., Metcalfe, D.D., 1999. Mast cell–T cell interactions. J. Allergy Clin. Immunol. 104, 517–523.

Muraro, A., Dreborg, S., Halken, S., Host, A., Niggemann, B., Aalberse, R., Arshad, S.H., Berg Av, A., Carlsen, K., Duschen, K., Eigenmann, P., Hill, D., Jones, C., Mellon, M., Oldeus, G., Oranje, A., Pascual, C., Prescott, S., Sampson, H., Svartengren, M., Vandenplas, Y., Wahn, U., Warner, J.A., Warner, J.O., Wickman, M., Zeiger, R.S., 2004. Dietary prevention of allergic diseases in infants and small children. Part I. Immunologic background and criteria for hypoallergenicity. Pediatr. Allergy Immunol. 15, 103–111.

Navarro, P., Barbis, D.P., Antczak, D., Butler, J.E., 1995. The complete cDNA and deduced amino acid sequence of equine IgE. Mol. Immunol. 32, 1–8.

Pawankar, R., Okuda, M., Yssel, H., Okumura, K., Ra, C., 1997. Nasal mast cells in perennial allergic rhinitics exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. J. Clin. Invest. 99, 1492–1499.

Pfeffer, A., Shaw, R.J., Green, R.S., Phegan, M.D., 2005. The transfer of maternal IgE and other immunoglobulins specific for Trichostrongylus colubriformis larval excretory/secretory product to the neonatal lamb. Vet. Immunol. Immunopathol. 108 (3–4), 315–323.

Pinto, L.A., Steudemann, L., Depner, M., Klopp, N., Illig, T., Weiland, S.K., von Mutius, E., Kabesch, M., 2007. STAT1 gene variations, IgE regulation and atopy. Allergy 62, 1456–1461.

Reddy, A., Fried, B., 2008. Atopic disorders and parasitic infections. Adv. Parasitol. 66, 149–191.

Russell, A.F., 1948. The development of helminthiasis in thouroughbred foals. J. Comp. Pathol. 58, 107–127.

Schnulle, P.M., Hurley, W.L., 2003. Sequence and expression of the FcRn in the porcine mammary gland. Vet. Immunol. Immunopathol. 91, 227–231.

Shimizu, A., Takahashi, N., Yaoita, Y., Honjo, T., 1982. Organization of the constant-region gene family of the mouse immunoglobulin heavy chain. Cell 28, 499–506.

Smits, H.H., Hartgers, F.C., Yazdanbakhsh, M., 2005. Helminth infections: protection from atopic disorders. Curr. Allergy Asthma Rep. 5, 42–50.

Staley, T.E., Bush, L.J., 1985. Receptor mechanisms of the neonatal intestine and their relationship to immunoglobulin absorption and disease. J. Dairy Sci. 68, 184–205.

Swain, P., Dash, S., Bal, J., Routray, P., Sahoo, P.K., Sahoo, S.K., Saurabh, S., Gupta, S.D., Meher, P.K., 2006. Passive transfer of maternal antibodies and their existence in eggs, larvae and fry of Indian major carp, *Labeo rohita* (Ham.). Fish Shellfish Immunol. 20, 519–527.

Thatcher, E.F., Gershwin, L.J., 1989. Colostral transfer of bovine immunoglobulin E and dynamics of serum IgE in calves. Vet. Immunol. Immunopathol. 20, 325–334.

Vernersson, M., Aveskogh, M., Hellman, L., 2004. Cloning of IgE from the echidna (*Tachyglossus aculeatus*) and a comparative analysis of epsilon chains from all three extant mammalian lineages. Dev. Comp. Immunol. 28, 61–75.

Vernersson, M., Aveskogh, M., Munday, B., Hellman, L., 2002. Evidence for an early appearance of modern post-switch immunoglobulin isotypes in mammalian evolution (II); cloning of IgE, IgG1 and IgG2 from a monotreme, the duck-billed platypus, *Ornithorhynchus anatinus*. Eur. J. Immunol. 32, 2145–2155.

Wagner, B., Flaminio, J.B., Hillegas, J., Leibold, W., Erb, H.N., Antczak, D.F., 2006. Occurrence of IgE in foals: evidence for transfer of maternal IgE by the colostrum and late onset of endogenous IgE production in the horse. Vet. Immunol. Immunopathol. 110, 269–278.

Wagner, B., Miller, D.C., Lear, T.L., Antczak, D.F., 2004. The complete map of the Ig heavy chain constant gene region reveals evidence for seven IgG isotypes and for IgD in the horse. J. Immunol. 173, 3230–3242.

Wagner, B., Radbruch, A., Rohwer, J., Leibold, W., 2003. Monoclonal anti-equine IgE antibodies with specificity for different epitopes on the immunoglobulin heavy chain of native IgE. Vet. Immunol. Immunopathol. 92, 45–60.

Warr, G.W., Magor, K.E., Higgins, D.A., 1995. IgY: clues to the origins of modern antibodies. Immunol. Today 16, 392–398.

Wen, H.J., Lin, Y.C., Lee, Y.L., Guo, Y.L., 2006. Association between cord blood IgE and genetic polymorphisms of interleukin-4, the beta-subunit of the high-affinity receptor for IgE, lymphotoxin-alpha, and tumor necrosis factor-alpha. Pediatr. Allergy Immunol. 17, 489–494.

West Jr., A.P., Herr, A.B., Bjorkman, P.J., 2004. The chicken yolk sac IgY receptor, a functional equivalent of the mammalian MHC-related Fc receptor, is a phospholipase A2 receptor homolog. Immunity 20, 601–610.

Westrom, B.R., Ohlsson, B.G., Svendsen, J., Tagesson, C., Karlsson, B.W., 1985. Intestinal transmission of macromolecules (BSA and FITC-dextran) in the neonatal pig: enhancing effect of colostrum, proteins and proteinase inhibitors. Biol. Neonate 47, 359–366.

Wilson, A.D., Badnell-Waters, A.J., Bice, R., Kelland, A., Harris, P.A., Nicol, C.J., 2007. The effects of diet on blood glucose, insulin, gastrin and the serum tryptophan: large neutral amino acid ratio in foals. Vet. J. 174, 139–146.

Wilson, A.D., Harwood, L., Torsteinsdottir, S., Marti, E., 2006. Production of monoclonal antibodies specific for native equine IgE and their application to monitor total serum IgE responses in Icelandic and non-Icelandic horses with insect bite dermal hypersensitivity. Vet. Immunol. Immunopathol. 112, 156–170.

Wilson, W.D., Mihalyi, J.E., Hussey, S., Lunn, D.P., 2001. Passive transfer of maternal immunoglobulin isotype antibodies against tetanus and influenza and their effect on the response of foals to vaccination. Equine Vet. J. 33, 644–650.

Yoshida, M., Masuda, A., Kuo, T.T., Kobayashi, K., Claypool, S.M., Takagawa, T., Kutsumi, H., Azuma, T., Lencer, W.I., Blumberg, R.S., 2006. IgG transport across mucosal barriers by neonatal Fc receptor for IgG and mucosal immunity. Springer Semin. Immunopathol. 28, 397–403.

Yu, L.C., Yang, P.C., Berin, M.C., Di Leo, V., Conrad, D.H., McKay, D.M., Satoskar, A.R., Perdue, M.H., 2001. Enhanced transepithelial antigen transport in intestine of allergic mice is mediated by IgE/CD23 and regulated by interleukin-4. Gastroenterology 121, 370–381.

Zhao, Y., Kacskovics, I., Rabbani, H., Hammarstrom, L., 2003. Physical mapping of the bovine immunoglobulin heavy chain constant region gene locus. J. Biol. Chem. 278, 35024–35032.