



Grayson-Jockey Club Research Foundation: Review Article

The development of equine immunity: Current knowledge on immunology in the young horse

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Summary

The development of equine immunity from the fetus to adulthood is complex. The foal's immune response and the immune mechanisms that they are equipped with, along with changes over the first months of life until the immune system becomes adult-like, are only partially understood. While several innate immune responses seem to be fully functional from birth, the onset of adaptive immune response is delayed. For some adaptive immune parameters, such as immunoglobulin (Ig)G1, IgG3, IgG5 and IgA antibodies, the immune response starts before or at birth and matures within 3 months of life. Other antibody responses, such as IgG4, IgG7 and IgE production, slowly develop within the first year of life until they reach adult levels. Similar differences have been observed for adaptive T cell responses. Interferon-gamma (IFN- γ) production by T helper 1 (Th1)-cells and cytotoxic T cells starts shortly after birth with low level production that gradually increases during the first year of life. In contrast, interleukin-4 (IL-4) produced by Th2-cells is almost undetectable in the first 3 months of life. These findings offer some explanation for the increased susceptibility of foals to certain pathogens such as *Rhodococcus equi*. The delay in Th-cell development and in particular Th2 immunity during the first months of life also provides an explanation for the reduced responsiveness of young horses to most traditional vaccines. In summary, all immune components of adult horses seem to exist in foals but the orchestrating and regulation of the immune response in immature horses is strikingly different. Young foals are fully competent and can perform certain immune responses but many mechanisms have yet to mature. Additional work is needed to improve our understanding of immunity and immune regulation in young horses, to identify the preferred immune pathways that they are using and ultimately provide new preventive strategies to protect against infectious disease.

Keywords: horse; foal; antibody; colostrum; cellular immunity

Introduction

Immune responses of neonatal and young foals are different from adults. Although basic immune components and mechanisms are similar in foals and adult horses, the regulation of immunity and responses to pathogens or vaccination are markedly different. Various specific immune parameters influence the different outcomes of immunity in young foals including reduced antibody [1–3] and T cell responses [4] as well as modified cytokine profiles to most pathogens and antigenic stimuli compared with adult horses [4–7]. The differences in the foal's responsiveness to immune stimulation have sometimes been interpreted as an 'immunodeficiency'. This conclusion can certainly be defended when comparing foal immunity to the quantitatively much higher adult immune response to the same antigens. However, similar differences between young and adult immunity have been observed in various mammalian species [8–11] suggesting that a reduced responsiveness to pathogens and immune stimulation early in life can be a necessary survival strategy of mammals and not a defect. Therefore, we recommend that the foal's immune system be considered immature but capable and not deficient.

Taking the evolutionary success of the horse into consideration, it can be assumed that there are good reasons for an immature immune response early in life. One reason could be the high load of novel antigens that the neonatal foal is exposed to after birth. Coming from the much protected, low antigen exposure *in utero*, the foal is born into an environment with high antigenic pressure. After birth, the neonatal immune system is

suddenly confronted with a wide variety of environmental antigens, commensal bacteria, parasites and sometimes virulent pathogens – all primary exposures. In a very short amount of time the foal's immune system has to learn to recognise pathogens but also tolerate commensals and harmless environmental and food antigens. In addition, the young foal's immune system has to manage a high degree of growth and cell proliferation during the first year of life. From an immunological perspective, the high environmental antigenic pressure after birth and demand for controlled cell proliferation while the young horse is growing both require very well established immune regulatory mechanisms. Effective immune regulation and tolerance are likely crucial mechanisms early in life to control inflammatory reactions and prevent malfunctioning of cell proliferation and growth. It is thus not surprising that the development of the young foal's immune system requires a high level of immune regulation and differs from the adult horse.

Significant problems arise when we try to protect foals from infectious diseases. Neonatal vaccination is a challenging task. Young foals respond poorly to available vaccine formulations, developed for adult horses, as indicated by delayed or weak adaptive immune responses to vaccination against different pathogens [12–19]. The design of vaccines that induce immunity and protection in very young foals likely requires a better understanding of the immune response of foals to relevant equine pathogens. The identification of specific mechanisms leading to robust immune responses early in life can result in new vaccination strategies that specifically trigger and optimise immunity in young foals.

TABLE 1: Immunoglobulin (Ig) nomenclature and functions in adult horses

Ig Isotype ^a		Basic immune function	Increased Ig isotypes after infection and/or association with protection from infectious diseases
Original nomenclature	Current designation		
IgM	IgM	Complement activation ^a	Primary immune response
IgD	IgD	Unknown	Unknown
IgGa	IgG1	Complement activation ^b :	IgG1 and IgG4/7: Influenza ^c , EHV-1 ^{d-e} , EHV-4 ^h , <i>R. equi</i> ^{i,j} IgG3/5: tetanus toxoid ^k , pneumococcus ^a , nematodes ^l , <i>Theileria equi</i> (IgG3) ^m
	IgG2	IgG1, IgG4/7, IgG3	
IgG(T)	IgG3	Fc-receptor binding ^b :	
IgGb	IgG4	IgG1, IgG4/7, IgG3/5	
IgG(T)	IgG5	Staphylococcal protein A binding ^b :	
IgGc	IgG6	IgG1 > IgG3 > IgG4 > IgG7 > IgG2 = IgG5 > IgG6	
IgGb	IgG7	Streptococcal protein G binding ^b :	
		IgG1 = IgG4/7 > IgG3 > IgG2 = IgG6	
IgE	IgE	Binding to high and low affinity IgE receptor on equine cells ⁿ	Parasite immunity ⁿ Allergic responses ⁿ
IgA	IgA	Neutralisation and IgA receptor binding ^o	Mucosal immunity ^{a,o}

References in table: ^a[24]; ^b[42]; ^c[26]; ^d[44]; ^e[45]; ^f[74]; ^g[75]; ^h[76]; ⁱ[27]; ^j[16]; ^k[25]; ^l[77]; ^m[78]; ⁿ[79]; ^o[80]. EHV-1 = equine herpesvirus-1.

What do we currently know about the immune response of foals? This review article will focus on the foal's immunity from birth to the first year of life, immune components in colostrum and milk, the onset of both cellular immunity and antibody production in healthy foals, including the effect of weaning on the immune system.

Colostrum/passive transfer

The epitheliochorial placentation of the mare prevents transfer of immunoglobulins (Igs) *in utero* to the fetus. Therefore, protection against environmental opportunistic pathogens is provided to the foal through the ingestion of Igs in the colostrum as soon after birth as possible. The Ig transport from the mammary gland to the colostrum and through the neonatal foal's gut is mediated by a transiently expressed specific IgG receptor, called neonatal Fc-receptor (FcRn), which is conserved throughout mammals [20,21]. As the ability of the foal to absorb intact Igs dissipates and the mare's colostrum turns to milk 12–24 h after foaling [22], timely passive transfer is important for the prevention of sepsis and neonatal survival [23]. An emphasis has been placed on Ig transfer. However, other maternal immune components such as maternal immune cells and cytokines, are also transferred to neonates with the colostrum and likely play significant roles in neonatal immune induction, protection against pathogens and in priming immune system development.

Antibodies in colostrum

Immunoglobulin G is the most abundant in equine colostrum while IgA is a minor colostrum component [1]. Horses have 7 different IgG isotypes with

different immunological functions [24]. The current IgG isotype nomenclature for the horse (IgG1–IgG7) is based on the designation of the Ig heavy chain constant region genes (*IGHG1–IGHG7*) [24]. The original and current Ig nomenclatures are outlined in Table 1. Compared with mare serum, almost all IgG isotypes, IgA and IgE are enriched in colostrum (Table 2). IgG4/7 represents the major subclass in serum of adult horses and in colostrum and has been suggested to play an essential role in protection against bacterial and viral infections [25–27]. However, IgG1, IgG3/5 and IgA are also substantially enriched in the colostrum (Table 2), can mediate important immune functions and contribute to the overall protection of the neonate from disease (Table 1). The importance of maternal antibody transfer has been thoroughly investigated [23,28] and many sick foals presented to neonatal intensive care units have partial or complete failure of passive transfer [23]. Vaccination of mares against various pathogens including, but not limited to, influenza, tetanus and rotavirus, increases the transfer of antigen-specific IgG to foals with the colostrum [15,28–30]. For diseases that can be prevented by pre-existing neutralising antibodies, like tetanus, sufficient concentrations of maternally derived antibodies are fully protective for the newborn foal. For intracellular pathogens, such as *Rhodococcus equi*, neutralising antibodies also have protective effects [27] but on their own are not fully protective.

Cytokines in colostrum

Cytokines are potent immune response modulators. Prior to colostrum ingestion and absorption, healthy neonatal foal serum does not contain detectable amounts of the cytokines interleukin-6 (IL-6) and tumour necrosis factor- α (TNF- α). However, they have been measured in equine colostrum and foal serum post suckling [31,32]. The concentrations of

TABLE 2: Normal immunoglobulin (Ig) concentrations (mg/ml) in adult horse and foal serum, colostrum and milk

	IgM	IgG1	IgG4/7 ^a	IgG3/5 ^a	IgG6	IgE	IgA
Adult serum	1.1 $\pm$ 0.4 ^{b,c}	3.4 $\pm$ 2.0 ^d	19.6 $\pm$ 6.5 ^d	4.0 $\pm$ 2.5 ^d	0.2 $\pm$ 0.1 ^d	0.08 $\pm$ 0.09 ^e	0.4 $\pm$ 0.3 ^d
Colostrum	1.23 $\pm$ 0.32 ^b	82.0 $\pm$ 44.0 ^d	183.0 $\pm$ 38.0 ^d	44.0 $\pm$ 25.0 ^d	0.3 $\pm$ 0.1 ^d	0.773 ^e	9.0 $\pm$ 3.0 ^d
Foal serum							
Presuckle	NA	0.03 $\pm$ 0.03 ^d	ND ^d	ND ^d	ND ^d	ND ^e	ND ^d
Day 1 p.p.	NA	10.0 $\pm$ 2.0 ^d	29.0 $\pm$ 3.6 ^d	4.8 $\pm$ 2.9 ^d	ND ^d	0.261 ^e	0.4 $\pm$ 0.1 ^d
Day 63 p.p.	NA	14.3 $\pm$ 5.0 ^d	8.5 $\pm$ 3.1 ^d	3.8 $\pm$ 1.4 ^d	ND ^d		0.1 $\pm$ 0.1 ^d
Milk ^d							
Day 1 p.p.	NA	1.6 $\pm$ 1.0	3.1 $\pm$ 1.6	0.8 $\pm$ 0.6	0.06 $\pm$ 0.06	NA	0.3 $\pm$ 0.1
Day 7 p.p.	NA	0.7 $\pm$ 0.3	0.9 $\pm$ 0.4	0.3 $\pm$ 0.2	ND	NA	0.7 $\pm$ 0.2
Day 14 p.p.	NA	0.4 $\pm$ 0.2	0.5 $\pm$ 0.3	0.2 $\pm$ 0.1	ND	NA	0.7 $\pm$ 0.2
Day 63 p.p.	NA	0.08 $\pm$ 0.1	0.1 $\pm$ 0.06	0.06 $\pm$ 0.04	ND	NA	0.6 $\pm$ 0.2

^aIgG4/7 represents the former IgGb subclass of the horse and IgG3/5 the former IgG(T) subclass. Monoclonal antibodies to IgG4/7 or IgG3/5 recognised both isotypes. Data were obtained from ^b[40]; ^c[81]; ^d[1]; ^e[3]. Concentrations are given in mean $\pm$ s.d.; NA = not available; ND = not detectable; p.p. = *post partum*.

these cytokines in foal serum post suckling vary widely with a 200- and 2500-fold difference between the minimum and maximum values for IL-6 and TNF- α , respectively. TNF- α was not associated with IgG concentrations in the post suckle foal serum [31,32]. However, IL-10 was not detected in either healthy mare serum or colostrum around the time of foaling [32]. With both TNF- α and IL-6, both inflammatory cytokines, being present in the colostrum and IL-10 being absent, these preliminary results suggest that cytokine transfer to the foal through colostrum is selective and promotes an inflammatory response, possibly to increase overall immune alertness directly after birth. However, the role maternal passively transferred cytokines play in neonatal immune development is currently poorly understood. In clinical cases interpretation of the cytokine concentrations must also consider passive transfer and not simply production by the foal in response to infection and/or inflammation.

Maternal cells in colostrum

Intestinal uptake of colostrum leucocytes has been documented in calves [33], lambs and pigs [34] and may occur in a similar manner in the foal. Maternal cow cells enter circulation and peak trafficking occurs 12–24 h after ingestion and cells disappear from the neonates' blood by 36 h post ingestion [35]. Maternal lymphocytes might migrate to secondary lymphoid tissues where they may provide direct protection to the neonate or initiate adaptive immune responses. Our laboratory recently described the phenotypic characteristics and cytokine secretion of colostrum immune cells and mare peripheral blood mononuclear cells (PBMC) at the time of foaling. Equine colostrum contains mostly CD4⁺ and CD8⁺ lymphocytes with a few B cells. CD8⁺ cytotoxic T cells were significantly enriched in the colostrum compared with mare PBMC. Colostrum T cells stimulated *in vitro* produced mainly interferon (IFN)- γ and IL-17 and only low amounts of IL-4 and IL-10, while stimulation of PBMC from the same mares resulted in higher IL-4, IL-10, similar IFN- γ and lower IL-17 production. The phenotypic and functional difference in the immune cells between the mare's blood and the colostrum suggests that cytotoxic T cells and IFN- γ and IL-17 producing Th-cell subsets are transferred to the colostrum in a selective process [36]. These new findings warrant further investigation of the potential roles of maternal T cells in providing passive cellular immunity, activating or modulating early immune recognition and perhaps immune development in neonatal foals.

Milk and local protection in gut – what do we know about milk in horses?

In addition to the dominating IgG antibodies, IgA is also present in equine colostrum as a minor component [1]. The transport mechanism for IgA into the mammary gland is currently unknown. Colostrum and milk IgA could be of maternal serum origin and/or be locally produced by plasma cells in the mammary gland. By 1–2 weeks *post partum*, the IgG concentrations in the milk decrease and IgA becomes the most abundant isotype in the mare's milk (Table 2). The colostrum IgA is absorbed and results in post suckle serum IgA in foals [1,2,37]. By about 3 weeks of age the maternal IgA has dissipated and the foal's serum concentration is low. Endogenous serum IgA production is detectable at about 1–2 months of age [1,2,38]. The milk IgA concentration remains constant at around 0.06–0.8 g/l depending on the reference cited [1,37] and is presumed to provide continuous local gastrointestinal passive protection.

Adaptive immunity

Foals at birth are immunologically naive to environmental antigens and thus lack adaptive immunity, such as preformed pathogen-specific antibodies, B and T cell memory and effector cells. Consequently, they are highly susceptible to respiratory and intestinal pathogens, like *R. equi*, rotavirus and others, many of which rarely occur in adults and only as opportunistic pathogens [16,23,38].

Antibody production in foals

Antibody production begins *in utero*. Neutralising antibodies are produced by the equine fetus when antigens are injected after 200 days of gestation

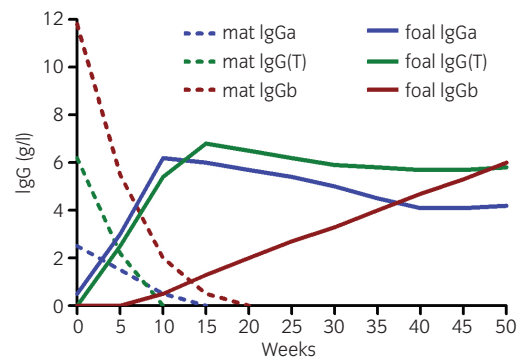


Fig 1: Maternal (mat) immunoglobulin (Ig)Gs and onset of IgG production in foals. The figure is based on data from Sheoran *et al.* (2000) and Holznagel *et al.* (2003) [1,2]. Antibodies were measured in foal serum before and after birth and in colostrum and data are accumulated in this figure. The onset of IgG1 (IgGa), IgG3/5 (IgG(T)) and IgG4/7 (IgGb) production was extrapolated based on the time points when these isotypes were first detectable on top of the residual maternal antibodies which were transferred to the foals with the colostrum. IgG1 is routinely detectable in serum of foals before colostrum uptake.

[39]. During normal pregnancy, the immune system of the equine fetus produces both IgM [40] and IgG1 [1] and therefore both isotypes are detected in serum from healthy neonates before they suckle colostrum (Table 2). The production of IgM and IgG by the foal continues after birth. However, the naïve immune system of the neonatal foal needs maternal support during the first weeks of life [23,41]. Nevertheless, the antibody profile of young foals shows certain differences from adult horses. Adult horse serum contains predominantly IgG4/7, with moderately high concentrations of IgG3/5 and IgG1. Initially, the Ig constituents of foal serum post colostrum suckling mirror those of the mare's colostrum. Then, the foal total serum immunoglobulin concentration declines as the maternally transferred IgGs are utilised and degrade with the half-life for IgG1, IgG4/7 and IgG3/5 of 18, 32 and 21 days, respectively [1]. Once the total immunoglobulin concentration in foal serum reaches a nadir, the subsequent increase in IgG isotypes is endogenous. However, what cannot be determined from this extrapolation is the exact time point at which the foal started producing IgG, except in the case of IgG1 which is produced before birth [1].

Studies comparing the immunoglobulin isotype composition in foal serum reported reduced IgG4/7 concentrations during the first year of life [1,2]. IgG4/7 is the subclass with the highest concentration in adult horse serum [1,2]. Foal endogenous IgG4/7 was first detectable by 16–20 weeks and increased slowly during the first year of life [2]. In contrast, endogenous IgG1, IgG3/5 and IgA were detectable in foal serum within the first 5–8 weeks of life. Foal IgG1 increased by 2–3 months to concentrations 3-fold higher in foal than in adult serum. Immunoglobulin G4/7 concentrations in foal serum were lower than those of IgG1 and IgG3/5 between 12 weeks and 8 months and were also still below adult IgG4/7 serum concentrations by one year of age [2]. However, the immune mechanisms mediated by IgG1, IgG4/7 and IgG3/5, such as complement activation, Fc-Rn interaction and binding to bacterial cell wall components, overlap widely [42] (Table 1). Therefore, the delay in IgG4/7 antibody production may be compensated functionally by the higher circulating IgG1 and adult-like IgG3/5 concentrations which are detectable as early as 2 months of age (Table 2, Fig 1). It should be emphasised that endogenous antibody production in foals was measured in all studies as an increase in addition to residual maternal antibodies and therefore, the true onset of antibody production in foals is earlier. Foal antibody production starts before birth for IgG1, shortly after birth for IgG3/5 and IgA and likely begins at around 5 weeks of age for IgG4/7 (Fig 1).

Immunoglobulin E is also enriched in and transferred with the colostrum and maternal IgE is detectable shortly after colostrum uptake in foal serum and on foal leucocytes. By 2–3 months of age, IgE or IgE positive cells are no longer detectable in the foal's circulation. Once maternal IgE concentrations wane, foals lack endogenous IgE in their serum and bound to IgE-receptor on the surface of white blood cells, including basophils for

a few months [3]. Endogenous IgE production was detected as early as 6 months of age in some Icelandic horses [43] and by 9–11 months of age in Thoroughbreds [3]. Although there is still no direct evidence for a link between the regulatory pathways that result in the synthesis of IgG4, IgG7 and IgE, the class switch recombination events leading to production of these 3 isotypes could be directly or indirectly suppressed for several months early in life whereas the immune mechanisms leading to IgM, IgG1, IgG3, IgG5 and IgA production seem to be fully intact.

In summary, neonatal and young foals are highly proficient in producing IgM, IgG1, IgG3, IgG5 and IgA antibodies but their production of IgG4, IgG7 and IgE isotypes is reduced and/or delayed compared with adult horses. Young foals preferentially produce certain isotypes such as IgG1, IgG3 and IgG5 and these isotypes are the quantitatively dominating IgGs in the serum between 3 and 8 months of age. Based on these findings, it can be concluded that B cell activation and class switching to IgG1, IgG3 and IgG5 occur, and that IgG1, IgG3 and IgG5 producing plasma cells develop during the first 2–3 months of life resulting in adult-like or even higher serum concentrations of these IgG isotypes. It is thus surprising that young foals mount negligible antibody titres after vaccination with approved horse vaccines and begs the question, what could cause the low antibody response of young foals to these vaccines?

Maternal antibody interference

Maternal antibodies have been implicated in the reduced antibody response to vaccination in foals less than 6 months of age [12,13,15]. However, control groups consisting of foals that did not receive maternal antibodies were lacking. There is only one study investigating foals that naturally suckled colostrum or received additional colostrum and included 2 foals that were colostrum-deprived. The serum fraction that primarily consists of antibodies, γ -globulin concentrations were similar in all groups from 2 to 12 months of age. The onset of γ -globulin production was comparable between groups and the ability to produce γ -globulin was estimated to begin very soon after birth. One of the colostrum-deprived foals developed joint swelling and hindlimb oedema at one month of age. This foal showed a rapid increase in serum γ -globulin which can be interpreted as an antibody response to infection, while the other colostrum-deprived foal showed a more steady increase of γ -globulin between 3 weeks and 4 months of age [28].

There are various studies that have measured influenza or tetanus-specific antibody responses in foals after vaccination. Foals born to vaccinated mares and that received maternal antibodies with colostrum were vaccinated with approved vaccines at different times after birth [12,13,15]. Repeated vaccination at 6 weeks, 10 weeks and 3–5 months of age produced no detectable serum antibodies to influenza antigens at 4 months after the last vaccination [13]. Most, but not all foals, responded to the vaccine when vaccinated first at 6–8 months of age and after maternal antibodies had virtually disappeared [13]. Vaccination of 2 foal groups at 12, 16 and 32 weeks or 24, 28 and 44 weeks of age with the same influenza vaccine resulted in a detectable antibody increase after the third vaccination and foals that were vaccinated using the latter schedule had higher and longer lasting antibody responses [12]. Similarly, vaccination of foals at 6 months of age with a different influenza vaccine and against tetanus induced an increase in antibodies, while vaccination starting at 3 months of age required several booster vaccinations until an antibody increase was measured [15]. Because these studies did not have control groups consisting of colostrum deprived foals or foals that received colostrum from mares that had not been vaccinated with the pathogen of interest, they do not provide sufficient evidence to support that maternal antibodies interfere with the antibody response to vaccination in foals. Although high concentrations of maternal antibodies could have some suppressive effect on B cell development in very young foals, the poor response to vaccination could also be related to the development of the immune system of the foal itself.

Considering the newer data showing that foals can produce IgG1 before and IgG3/5 antibodies shortly after birth and mount total IgG1 and IgG3/5 serum concentrations by 2–3 months of age that are higher or as high as those in adult horses [1,2], the reduced antibody response to vaccination in young foals could also reflect a more complex difference in the foal's immune response to specific vaccine formulations. Almost all approved vaccines have been optimised to induce immunity in adults and do not

take the specific properties of the neonatal immune system into account. For example, various groups have reported that certain cytokine and Th-cell responses are reduced in young foals (see below) [4–6]. These cellular immune responses are, however, important to induce B cell activation and antibody production. It also seems to be discrepant that pre-existing maternal antibodies in foals should be able to inhibit antibody production, while pre-existing antibodies in an adult horse do not interfere with vaccination as long as the adult horses are not hypervaccinated. In other words, a vaccine boost in a normal adult horse will result in an antibody increase in addition to pre-existing antibodies to the same pathogen [44,45]. Very likely the reasons for the low antibody response of young foals to traditional vaccine formulations, are more complex than just a passive or inhibitory interference of maternal Ig with endogenous antibody production and differences in adult and foal T cell responses may contribute to the poor outcomes of vaccinating young foals.

T cell immunity

Cytokines produced by different Th-cell subsets play major roles in regulating adaptive immune response to infectious disease and vaccination and cytokine expression in foals during the first year of life differs from adults [4,6,7,16]. Specifically, T cell and cytokine responses are reduced and delayed in foals for 3 months to one year after birth [4,6,46]. Despite the overall quantitatively reduced cytokine response compared with adult horses, cellular immune responses of young foals are Th1-biased and Th2 responses are barely detectable for an extended time after birth [4]. Interferon- γ production by Th1 and cytotoxic T cells is detectable a few days after birth and increases gradually with age [4,6] (Figs 2a and c). In contrast, IL-4 production by Th2 cells is almost absent for at least the first 3 months of life [4] (Figs 2b and c). Since Th2-dependent B cell activation is a major pathway in adults to induce high antibody concentrations in response to vaccination, the virtually absent Th2 pathway in young foals provides another possible explanation for the low responsiveness of foals to classical vaccines. As outlined in the previous section, foals produce high amounts of IgG1 and IgG3/5 early in life while they are still responding poorly to conventional vaccination. It is thus likely that Th-independent immune mechanisms (alternative pathways) are important for the observed IgG antibody production early in the foal's life.

There is some controversy in the current equine literature about Th-cell responses of the neonatal and young foal. Some authors suggest that foals are IFN- γ deficient [6] or that the immune system exhibits a Th2-bias [5,17,47]. However, foal Th1 and Th2 cells were not determined in these studies. While the total IFN- γ production in foals is certainly decreased compared with adults, the conclusion on a Th2-biased immune response might have been extrapolated from a paradigm in neonatal mice. Murine neonates have a Th2 dominated immune response, although strong Th1 responses can be induced depending on the experimental conditions [48,49]. However, not all mammalian species are the same. Data from human neonatal T cells does not support the conclusion that T cells are Th2 polarised and rather point towards a Th1 or Th0 type response in human neonates [50,51]. Only one study has compared neonatal and young foal Th1- and Th2-cell responses by actually evaluating intracellular IFN- γ and IL-4 in T cell subsets and not just by measuring overall cytokine production in PBMC. This work showed that young foals have an impaired Th2 response, are Th1 biased, can produce IFN- γ via Th1 and CD8⁺ cytotoxic T cells and that regulatory IL-10 production by T cells is developmentally mature in foals [4]. Thus, the discrepant conclusions on Th-cell immunity in foals in the equine literature are likely due to the fact that most available studies measured cytokine mRNA expression or the response of a single cytokine, most frequently IFN- γ , without taking the originating cell types or both, Th1 and Th2, responses into account. Cytokines can be produced by a variety of immune cells, not just Th-cells. For example, IFN- γ can be secreted by Th1-cells, cytotoxic T cells [4,46,52,53], $\gamma\delta$ T cells [54] or by innate immune cells such as natural killer cells [55] and possibly other cell types depending on their activation status. Interleukin-4 is produced by Th2-cells [56] but also in high concentrations by activated basophils and mast cells [57–60]. In the peripheral blood of healthy neonatal foals, an activated basophil population secreting high

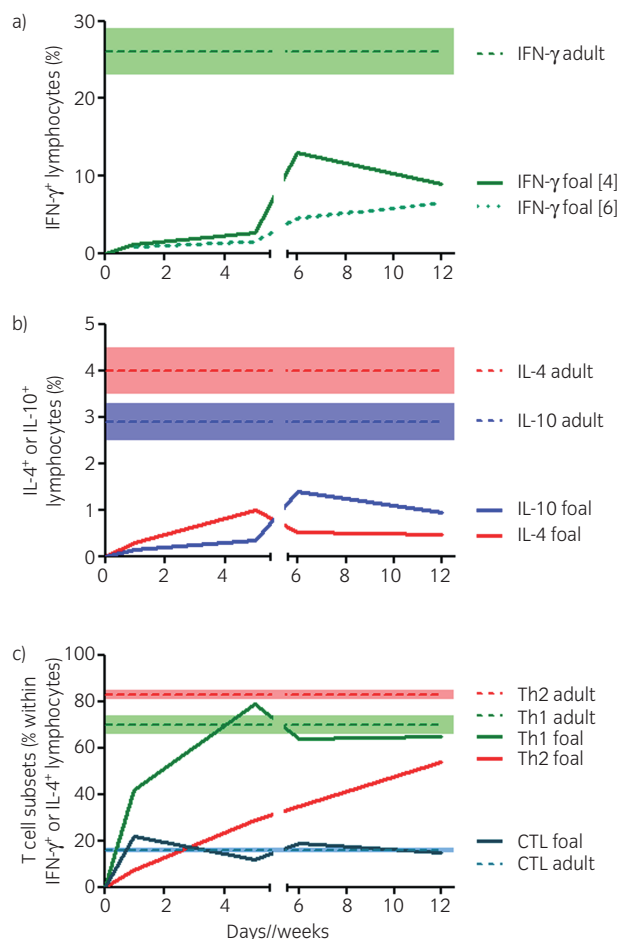


Fig 2: Cytokine and T cell responses in foals during the first 3 months of life. The data are based on Wagner *et al.* (2010) [4] and for total interferon (IFN)- γ producing cells also on Breathnach *et al.* (2006) [6]. Percentages of IFN- γ , interleukin (IL)-4 and IL-10 producing lymphocytes, T helper 1 (Th1) (IFN- γ + /CD4+), Th2-cells (IL-4 + /CD4+) and IFN- γ -producing cytotoxic T cells (CTL; IFN- γ + /CD8+) within the IFN- γ or IL-4 producing lymphocytes can be determined in foal and adult peripheral blood mononuclear cells (PBMC) after stimulation with PMA and ionomycin *in vitro*. This method leads to polyclonal activation of the cells and can be seen as their maximal capacity to produce cytokines by respective T cell subsets. After activation, cells are fixed, stained with cytokine and T cell markers and measured by flow cytometry. The analysis gate has been set on lymphocytes. a) Percentage of IFN- γ producing lymphocytes in Thoroughbred and Warmblood foals [4] and mixed-breed foals [6] expressed as means during the first 3 months of life. The split x-axis shows days (left half) or weeks (right half) of age. The dotted horizontal line gives the mean percentage of IFN- γ producing lymphocytes in adult horses. The shade below and above the adult mean value shows the corresponding standard error. b) Means of percentage of IL-4 and IL-10 producing lymphocytes during the first 3 months of life. The dotted lines and shades give the mean and standard error values for adult horses. Based on existing knowledge and shown in the upper 2 figures, IFN- γ , IL-4 and IL-10 are inducible in a lower percentage of lymphocytes in young foals than in adult horses. c) The percentage of IFN- γ producing lymphocytes was set for each time point as 100%. Within the IFN- γ -producing cells the proportion of Th1 and IFN- γ -producing cytotoxic T cells were analysed. Despite being overall quantitatively lower, the proportion of IFN- γ producing Th1 and CTL subsets is adult-like by Day 5 after birth. The same was done for Th2-cells within the IL-4 producing lymphocytes. Unlike Th1-cells and CTLs, the percentage of foal Th2-cells within the IL-4 producing lymphocytes slowly increase with age and by 3 months of age is still below the mean adult value.

concentrations of IL-4 is routinely detectable after birth [7]. Interleukin-4 expression by foal basophils may have been picked up in mRNA expression studies and was then mistakenly interpreted as a Th2 response of foals. Nevertheless, it can be postulated that the basophil-derived IL-4 has an immune stimulatory function early in the foal's life. The latter statement still requires experimental proof in foals. More recent data from mice

provide evidence that B cell activation and antibody production can be effectively initiated by innate immune cells including mast cells and basophils [61].

Despite the obvious differences in cellular immune responses between foals and adult horses, the majority of the foals in the horse population are healthy. This suggests that their developing immune system is able to adequately respond to almost all environmental exposures. However, foals are more vulnerable to certain pathogens and their immature immune system is likely one reason for the increased susceptibility to infection. Two foal diseases i.e. *R. equi* and equine herpesvirus-1 (EHV-1) serve as examples to outline the differences between foal and adult horse immunity and disease outcomes.

The inability of foals to respond to infection with adult-like T cell responses and especially with quantitatively comparable IFN- γ production, has been put forward as the reason for the increased susceptibility of foals to intracellular pathogens such as *R. equi* [5,6,18,62]. The ability of *R. equi* to cause severe pneumonia depends on a virulence plasmid within the bacteria. Infection of foals with a plasmid-cured *R. equi* derivative or the virulence plasmid-containing *R. equi* strain showed that the products of the virulence plasmid increase inflammatory cytokines mRNA expression in the lung tissue and decrease local IFN- γ mRNA expression in lung CD4+ cells [63]. Infection with *R. equi* can cause severe respiratory disease in some foals but minimal or no clinical signs in adult horses which generally mount a higher IFN- γ response to this opportunistic pathogen in both their Th1 and cytotoxic T cell populations [52]. However, foal innate immune cells, such as monocyte-derived dendritic cells, respond to infection with virulent *R. equi* with increased IL-12 mRNA expression compared with cells from adult horses [64]. Foal PBMC also showed a clear upregulation of IFN- γ mRNA, decreased IL-4 gene expression and greater IFN- γ /IL-4 transcript ratios in response to *R. equi* infection compared with adult horses [65], which is in agreement with the finding that Th-cells of young foals can generally produce IFN- γ but have a substantially reduced capacity to develop into IL-4 producing Th2-cells [4]. The increased innate immune response, the intact IFN- γ biased *R. equi*-specific response and overall Th1-biased immune response of young foals may offer an explanation for the observation that many foals develop only mild or subclinical disease after exposure to *R. equi*.

Interferon- γ production in response to infection with another intracellular pathogen, EHV-1, was also almost undetectable in foals but increased with age [46]. Equine herpesvirus-1 infection causes transient fever, depression and mild respiratory disease, while severe outcomes or neurological signs are rarely seen in foals. Most adult horses have some level of immunity to EHV-1 infection because of either previous infection and/or vaccination. Nevertheless, more severe outcomes including fatal neurological disease can occur in adult horses [66,67]. After EHV-1 infection, IFN- γ -producing cells in foals and young horses were identified as mainly CD8+ cytotoxic T cells. In older horses, the EHV-1 specific T cell response shifted towards a CD8+ phenotype resulting in a clear decrease in IFN- γ producing EHV-1-specific cytotoxic T cells [46]. Overall, the phenotypic variations in IFN- γ producing effector T cells are believed to offer some explanation for age-dependent differences in susceptibility to severe clinical disease induced by *R. equi* in foals or EHV-1 in adult horses. These 2 examples indicate that cytokine production and T cell development in foals vary depending on the pathogen. Because the cytokine network is complex and interactions between immune cells can be manifold, a better understanding of cytokine production by different innate and adaptive immune cells, their functional effects and contribution to equine immunity is required for many diseases of horses and foals to improve our ability to protect them from disease and fatal outcomes.

Innate immunity

While innate immune responses have not yet been systematically investigated in foals, some information has been accumulated. Foal neutrophils have a reduced phagocytic ability during the first 3 weeks and a decreased killing capacity during the first 3 months of life but, thereafter, foal neutrophils performed these functions equal to or better than those of adult horses [68]. Phagocytosis of bacteria or yeast by foal neutrophils required opsonisation by IgG and sufficient colostrum uptake supported an

increased opsonic capacity, while decreased maternal IgG4/7 levels in foal serum reduced opsonisation and neutrophil phagocytosis [69]. Cytokine expression studies in response to different stimuli show that some cytokines are downregulated and others are unchanged or upregulated compared with adult horses. For example, neonatal PBMC expressed less transforming growth factor (TGF)- β and IL-1 α mRNA transcripts in comparison with cells from adult horses [5]. Other innate cytokines such as IL-8, IL-12 and IL-23 were found to be increased or similarly expressed in PBMC from neonates compared with older foals [62]. Stimulation of PBMC from foals with lipopolysaccharide (LPS) and IFN- γ also resulted in increased IL-10 mRNA expression compared with PBMC from adult horses [70]. In contrast, neonatal foal PBMC secreted lower concentrations of IFN- α and IL-10 after *in vitro* infection with EHV-1 than PBMC from their mares [71].

Recent results have shown that neonatal innate immune cells are important producers of cytokines. In neonatal foals, high levels of IL-4 are produced by basophils during the first week after birth [7] while a Th2 response is virtually absent [4]. Interleukin-4 is required to induce long-lasting B cell memory responses and IgG antibodies. In neonatal foals, IL-4 produced by basophils may provide the stimulus that replaces the missing Th2 response by directly stimulating antibody production. This could offer an explanation for the early-in-life production of IgG1, IgG3 and IgG5 antibodies discussed above. The current glimpses into innate immune pathways indicate that the immature immune system of the foal uses certain immune pathways preferentially and that innate immune mechanisms may have more prominent roles than in adults by orchestrating immune responses and induction of immunity in very young foals.

Overall, the accumulated knowledge on cellular immunity in foals is still incomplete. Existing data suggests that 1) innate immune cells of foals, such as dendritic cells, basophils and likely others, may have important regulatory roles early in life, 2) total cytokine expression values induced by mitogenic or nonspecific stimulation should be evaluated with care because they may not correlate with cytokine responses after stimulation with specific pathogens and 3) despite the immaturity of the foal's adaptive immune system, most foals in the population are healthy and foals seem to be able to respond to some pathogens better than adult horses (for example, despite frequent infection, severe neurological disease induced by EHV-1 is rarely observed in foals).

Weaning and the immune system

At the time of weaning, foals are not only separated from their dam but often have a change in environment, diet and social changes which are highly stressful and lead to increases in plasma cortisol [72]. The effect of increased plasma cortisol or weaning on the immune system has not been extensively studied in horses. Foals weaned abruptly produced less intracellular IFN- γ and had reduced mRNA expression of IFN- γ , TNF- α and IL-10 between 7 and 14 days post weaning [73]. This suggests that the cell-mediated immune response in foals at the time of weaning is reduced and thus weaning is a time of increased susceptibility to infectious diseases.

Conclusions

The neonatal foal acquires Ig, T cells and cytokines from the dam through the colostrum which are crucial for health through the neonatal period, and possibly initiates the development of the immune system after birth. The foal's naïve yet very capable immune system has many environmental triggers and it must balance its response – recognising friend or foe; commensal or pathogen; tolerate or eliminate. For many infectious diseases, we need a more thorough investigation of the host immune response and the parameters that correlate with protective immunity. Understanding that foal immunity differs from the adult horse and learning the distinct immune mechanisms utilised by young horses will help us to develop preventative strategies for infectious diseases in early life.

Authors' declaration of interests

The Grayson-Jockey Club Research Foundation (GJCRF) commissioned this article as part of a series summarising areas relating to their priorities for research funding. The authors and other workers at their institute hold current and previous research grants funded by the GJCRF and the authors have served on GJCRF advisory committees.

Sources of funding

The authors thank the Harry M. Zweig Memorial Fund for Equine Research at Cornell University for their support of our projects on neonatal immunity.

Acknowledgements

The authors would like to thank Paula Sharp for her assistance with the manuscript preparation.

Authorship

Both authors contributed to the preparation and final approval of the manuscript.

References

1. Sheoran, A.S., Timoney, J.F., Holmes, M.A., Karzenski, S.S. and Crisman, M.V. (2000) Immunoglobulin isotypes in sera and nasal mucosal secretions and their neonatal transfer and distribution in horses. *Am. J. Vet. Res.* **61**, 1099-1105.
2. Holznagel, D.L., Hussey, S., Mihalyi, J.E., Wilson, W.D. and Lunn, D.P. (2003) Onset of immunoglobulin production in foals. *Equine Vet. J.* **35**, 620-622.
3. Wagner, B., Flaminio, J.B., Hillegas, J., Leibold, W., Erb, H.N. and 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.
4. Wagner, B., Burton, A. and Ainsworth, D. (2010) Interferon-gamma, interleukin-4 and interleukin-10 production by T helper cells reveals intact Th1 and regulatory TR1 cell activation and a delay of the Th2 cell response in equine neonates and foals. *Vet. Res.* **41**, 47-60.
5. Boyd, N.K., Cohen, N.D., Lim, W.S., Martens, R.J., Chaffin, M.K. and Ball, J.M. (2003) Temporal changes in cytokine expression of foals during the first month of life. *Vet. Immunol. Immunopathol.* **92**, 75-85.
6. Breathnach, C.C., Sturgill-Wright, T., Stiltner, J.L., Adams, A.A., Lunn, D.P. and Horohov, D.W. (2006) Foals are interferon gamma-deficient at birth. *Vet. Immunol. Immunopathol.* **112**, 199-209.
7. Wagner, B., Stokol, T. and Ainsworth, D.M. (2010) Induction of interleukin-4 production in neonatal IgE+ cells after crosslinking of maternal IgE. *Dev. Comp. Immunol.* **34**, 436-444.
8. Chase, C.C., Hurley, D.J. and Reber, A.J. (2008) Neonatal immune development in the calf and its impact on vaccine response. *Vet. Clin. N. Am.: Food Anim. Pract.* **24**, 87-104.
9. Butler, J.E., Zhao, Y., Sinkora, M., Wertz, N. and Kacsokovics, I. (2009) Immunoglobulins, antibody repertoire and B cell development. *Dev. Comp. Immunol.* **33**, 321-333.
10. Hodgins, D.C. and Shewen, P.E. (2012) Vaccination of neonates: problem and issues. *Vaccine* **30**, 1541-1559.
11. Ghazal, P., Dickinson, P. and Smith, C.L. (2013) Early life response to infection. *Curr. Opin. Infect. Dis.* **26**, 213-218.
12. van Maanen, C., Bruin, G., de Boer-Luijtz, E., Smolders, G. and de Boer, G.F. (1992) Interference of maternal antibodies with the immune response of foals after vaccination against equine influenza. *Vet. Q.* **14**, 13-17.
13. Van Oirschot, J.T., Bruin, G., de Boer-Luytze, E. and Smolders, G. (1991) Maternal antibodies against equine influenza virus in foals and their interference with vaccination. *Zentralbl. Veterinarmed. B* **38**, 391-396.
14. Varga, J., Fodor, L., Rusvai, M., Soos, I. and Makrai, L. (1997) Prevention of Rhodococcus equi pneumonia of foals using two different inactivated vaccines. *Vet. Microbiol.* **56**, 205-212.

15. Wilson, W.D., Mihalyi, J.E., Hussey, S. and 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.
16. Lopez, A.M., Hines, M.T., Palmer, G.H., Knowles, D.P., Alperin, D.C. and Hines, S.A. (2003) Analysis of anamnestic immune responses in adult horses and priming in neonates induced by a DNA vaccine expressing the vapA gene of *Rhodococcus equi*. *Vaccine* **21**, 3815-3825.
17. Mealey, R.H., Stone, D.M., Hines, M.T., Alperin, D.C., Littke, M.H., Leib, S.R., Leach, S.E. and Hines, S.A. (2007) Experimental *Rhodococcus equi* and equine infectious anemia virus DNA vaccination in adult and neonatal horses: effect of IL-12, dose, and route. *Vaccine* **25**, 7582-7597.
18. Dawson, T.R., Horohov, D.W., Meijer, W.G. and Muscatello, G. (2010) Current understanding of the equine immune response to *Rhodococcus equi*. An immunological review of *R. equi* pneumonia. *Vet. Immunol. Immunopathol.* **135**, 1-11.
19. Bresgen, C., Lammer, M., Wagner, B., Osterrieder, N. and Damiani, A.M. (2012) Serological responses and clinical outcome after vaccination of mares and foals with equine herpesvirus type 1 and 4 (EHV-1 and EHV-4) vaccines. *Vet. Microbiol.* **160**, 9-16.
20. Roopenian, D.C. and Akilesh, S. (2007) FcRn: the neonatal Fc receptor comes of age. *Nat. Rev. Immunol.* **7**, 715-725.
21. Cervenak, J. and Kacsokovics, I. (2009) The neonatal Fc receptor plays a crucial role in the metabolism of IgG in livestock animals. *Vet. Immunol. Immunopathol.* **128**, 171-177.
22. Jeffcott, L.B. (1971) Duration of permeability of the intestine to macromolecules in the newly-born foal. *Vet. Rec.* **88**, 340-341.
23. McGuire, T.C., Crawford, T.B., Hallowell, A.L. and Macomber, L.E. (1977) Failure of colostral immunoglobulin transfer as an explanation for most infections and deaths of neonatal foals. *J. Am. Vet. Med. Ass.* **170**, 1302-1304.
24. Wagner, B. (2006) Immunoglobulins and immunoglobulin genes of the horse. *Dev. Comp. Immunol.* **30**, 155-164.
25. McGuire, T.C., Crawford, T.B. and Henson, J.B. (1973) The isolation, characterisation and functional properties of equine immunoglobulin classes and subclasses. In: *Proceedings of the 3rd International Conference on Equine Infectious Diseases*, Eds: J.T. Bryans and H. Gerber, S. Karger, Basel, Switzerland. pp 364-381.
26. Nelson, K.M., Schram, B.R., McGregor, M.W., Sheoran, A.S., Olsen, C.W. and Lunn, D.P. (1998) Local and systemic isotype-specific antibody responses to equine influenza virus infection versus conventional vaccination. *Vaccine* **16**, 1306-1313.
27. Lopez, A.M., Hines, M.T., Palmer, G.H., Alperin, D.C. and Hines, S.A. (2002) Identification of pulmonary T-lymphocyte and serum antibody isotype responses associated with protection against *Rhodococcus equi*. *Clin. Diagn. Lab. Immunol.* **9**, 1270-1276.
28. Jeffcott, L.B. (1974) Studies on passive immunity in the foal. 1. Gamma-globulin and antibody variations associated with the maternal transfer of immunity and the onset of active immunity. *J. Comp. Pathol.* **84**, 93-101.
29. Sheoran, A.S., Karzenski, S.S., Whalen, J.W., Crisman, M.V., Powell, D.G. and Timoney, J.F. (2000) Prepartum equine rotavirus vaccination inducing strong specific IgG in mammary secretions. *Vet. Rec.* **146**, 672-673.
30. Powell, D.G., Dwyer, R.M., Traub-Dargatz, J.L., Fulker, R.H., Whalen, J.W., Jr, Srinivasappa, J., Acree, W.M. and Chu, H.J. (1997) Field study of the safety, immunogenicity, and efficacy of an inactivated equine rotavirus vaccine. *J. Am. Vet. Med. Ass.* **211**, 193-198.
31. Secor, E.J., Matychak, M.B. and Felipe, M.J. (2012) Transfer of tumour necrosis factor-alpha via colostrum to foals. *Vet. Rec.* **170**, 51-54.
32. Burton, A.B., Wagner, B., Erb, H.N. and Ainsworth, D.M. (2009) Serum interleukin-6 (IL-6) and IL-10 concentrations in normal and septic neonatal foals. *Vet. Immunol. Immunopathol.* **132**, 122-128.
33. Liebler-Tenorio, E.M., Riedel-Caspari, G. and Pohlenz, J.F. (2002) Uptake of colostral leukocytes in the intestinal tract of newborn calves. *Vet. Immunol. Immunopathol.* **85**, 33-40.
34. Tuboly, S. and Bernath, S. (2002) Intestinal absorption of colostral lymphoid cells in newborn animals. *Adv. Expt. Med. Biol.* **503**, 107-114.
35. Reber, A.J., Lockwood, A., Hippen, A.R. and Hurley, D.J. (2006) Colostrum induced phenotypic and trafficking changes in maternal mononuclear cells in a peripheral blood leukocyte model for study of leukocyte transfer to the neonatal calf. *Vet. Immunol. Immunopathol.* **109**, 139-150.
36. Perkins, G.A., Goodman, L.B., Wimer, C., Freer, H., Babayan, S. and Wagner, B. (2014) Maternal T-lymphocytes in equine colostrum express a primarily inflammatory phenotype. *Vet. Immunol. Immunopathol.* **161**, 141-150.
37. Jenvey, C., Caraguel, C., Howarth, G.B. and Riley, C.B. (2012) Identification of periparturient mare and foal associated predictors of post parturient immunoglobulin A concentrations in Thoroughbred foals. *Equine Vet. J.* **44**, Suppl. **43**, 73-77.
38. Galan, J.E., Timoney, J.F. and Lengemann, F.W. (1986) Passive transfer of mucosal antibody to *Streptococcus equi* in the foal. *Infect. Immun.* **54**, 202-206.
39. Martin, B.R. and Larson, K.A. (1973) Immune response of equine fetus to coliphage T2. *Am. J. Vet. Res.* **34**, 1363-1364.
40. McGuire, T.C. and Crawford, T.B. (1973) Passive immunity in the foal: measurement of immunoglobulin classes and specific antibody. *Am. J. Vet. Res.* **34**, 1299-1303.
41. Jeffcott, L.B. (1972) Passive immunity and its transfer with special reference to the horse. *Biol. Rev. Camb. Philos. Soc.* **47**, 439-464.
42. Lewis, M.J., Wagner, B. and Woof, J.M. (2008) The different effector function capabilities of the seven equine IgG subclasses have implications for vaccine strategies. *Mol. Immunol.* **45**, 818-827.
43. Marti, E., Ehrensperger, F., Burger, D., Ousey, J., Day, M.J. and Wilson, A.D. (2009) Maternal transfer of IgE and subsequent development of IgE responses in the horse (*Equus caballus*). *Vet. Immunol. Immunopathol.* **127**, 203-211.
44. Goodman, L.B., Wagner, B., Flaminio, M.J., Sussman, K.H., Metzger, S.M., Holland, R. and Osterrieder, N. (2006) Comparison of the efficacy of inactivated combination and modified-live virus vaccines against challenge infection with neuropathogenic equine herpesvirus type 1 (EHV-1). *Vaccine* **24**, 3636-3645.
45. Goehring, L.S., Wagner, B., Bigbie, R., Hussey, S.B., Rao, S., Morley, P.S. and Lunn, D.P. (2010) Control of EHV-1 viremia and nasal shedding by commercial vaccines. *Vaccine* **28**, 5203-5211.
46. Paillot, R., Daly, J.M., Luce, R., Montosso, F., Davis-Poynter, N., Hannant, D. and Kydd, J.H. (2007) Frequency and phenotype of EHV-1 specific, IFN-gamma synthesising lymphocytes in ponies: the effects of age, pregnancy and infection. *Dev. Comp. Immunol.* **31**, 202-214.
47. Morein, B., Blomqvist, G. and Hu, K. (2007) Immune responsiveness in the neonatal period. *J. Comp. Pathol.* **137**, Suppl. **1**, S27-S31.
48. Forsthuber, T., Yip, H.C. and Lehmann, P.V. (1996) Induction of TH1 and TH2 immunity in neonatal mice. *Science* **271**, 1728-1730.
49. Garcia, A.M., Fadel, S.A., Cao, S. and Sarzotti, M. (2000) T cell immunity in neonates. *Immunol. Res.* **22**, 177-190.
50. Delespesse, G., Yang, L.P., Ohshima, Y., Demeure, C., Shu, U., Byun, D.G. and Sarfati, M. (1998) Maturation of human neonatal CD4+ and CD8+ T lymphocytes into Th1/Th2 effectors. *Vaccine* **16**, 1415-1419.
51. Adkins, B. (1999) T cell function in newborn mice and humans. *Immunol. Today* **20**, 330-335.
52. Hines, S.A., Stone, D.M., Hines, M.T., Alperin, D.C., Knowles, D.P., Norton, L.K., Hamilton, M.J., Davis, W.C. and McGuire, T.C. (2003) Clearance of virulent but not avirulent *Rhodococcus equi* from the lungs of adult horses is associated with intracytoplasmic gamma interferon production by CD4+ and CD8+ T lymphocytes. *Clin. Diagn. Lab. Immunol.* **10**, 208-215.
53. Paillot, R., Daly, J.M., Juillard, V., Minke, J.M., Hannant, D. and Kydd, J.H. (2005) Equine interferon gamma synthesis in lymphocytes after in vivo infection and in vitro stimulation with EHV-1. *Vaccine* **23**, 4541-4551.
54. Kaufmann, S.H. (2002) Protection against tuberculosis: cytokines, T cells, and macrophages. *Ann. Rheum. Dis.* **61**, Suppl. **2**, ii54-ii58.
55. Farrar, M.A. and Schreiber, R.D. (1993) The molecular cell biology of interferon-gamma and its receptor. *Annu. Rev. Immunol.* **11**, 571-611.
56. Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* **7**, 145-173.
57. Wedemeyer, J., Tsai, M. and Galli, S.J. (2000) Roles of mast cells and basophils in innate and acquired immunity. *Curr. Opin. Immunol.* **12**, 624-631.
58. Min, B., Prout, M., Hu-Li, J., Zhu, J., Jankovic, D., Morgan, E.S., Urban, J.F., Jr, Dvorak, A.M., Finkelman, F.D., LeGros, G. and Paul, W.E. (2004) Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J. Expt. Med.* **200**, 507-517.
59. Gibbs, B.F. (2005) Human basophils as effectors and immunomodulators of allergic inflammation and innate immunity. *Clin. Expt. Med.* **5**, 43-49.
60. Sokol, C.L., Barton, G.M., Farr, A.G. and Medzhitov, R. (2008) A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* **9**, 310-318.
61. Vinuesa, C.G. and Chang, P.P. (2013) Innate B cell helpers reveal novel types of antibody responses. *Nat. Immunol.* **14**, 119-126.

62. Liu, T., Nerren, J., Liu, M., Martens, R. and Cohen, N. (2009) Basal and stimulus-induced cytokine expression is selectively impaired in peripheral blood mononuclear cells of newborn foals. *Vaccine* **27**, 674-683.
63. Giguere, S., Wilkie, B.N. and Prescott, J.F. (1999) Modulation of cytokine response of pneumonic foals by virulent *Rhodococcus equi*. *Infect. Immun.* **67**, 5041-5047.
64. Flaminio, M.J., Nydam, D.V., Marquis, H., Matychak, M.B. and Giguere, S. (2009) Foal monocyte-derived dendritic cells become activated upon *Rhodococcus equi* infection. *Clin. Vaccine Immunol.* **16**, 176-183.
65. Jacks, S., Giguere, S., Crawford, P.C. and Castleman, W.L. (2007) Experimental infection of neonatal foals with *Rhodococcus equi* triggers adult-like gamma interferon induction. *Clin. Vaccine Immunol.* **14**, 669-677.
66. Lunn, D.P., Davis-Poynter, N., Flaminio, M.J., Horohov, D.W., Osterrieder, K., Pusterla, N. and Townsend, H.G. (2009) Equine herpesvirus-1 consensus statement. *J. Vet. Intern. Med.* **23**, 450-461.
67. Kydd, J.H., Slater, J., Osterrieder, N., Lunn, D.P., Antczak, D.F., Azab, W., Balasuriya, U., Barnett, C., Brosnahan, M., Cook, C., Damiani, A., Elton, D., Frampton, A., Gilkerson, J., Goehring, L., Horohov, D., Maxwell, L., Minke, J., Morley, P., Nauwynck, H., Newton, R., Perkins, G., Pusterla, N., Soboll-Hussey, G., Traub-Dargatz, J., Townsend, H., Van de Walle, G.R. and Wagner, B. (2012) Third International Havemeyer Workshop on Equine Herpesvirus type 1. *Equine Vet. J.* **44**, 513-517.
68. Demmers, S., Johannisson, A., Grondahl, G. and Jensen-Waern, M. (2001) Neutrophil functions and serum IgG in growing foals. *Equine Vet. J.* **33**, 676-680.
69. Grondahl, G., Sternberg, S., Jensen-Waern, M. and Johannisson, A. (2001) Opsonic capacity of foal serum for the two neonatal pathogens *Escherichia coli* and *Actinobacillus equuli*. *Equine Vet. J.* **33**, 670-675.
70. Sponseller, B.A., de Macedo, M.M., Clark, S.K., Gallup, J.M. and Jones, D.E. (2009) Activation of peripheral blood monocytes results in more robust production of IL-10 in neonatal foals compared to adult horses. *Vet. Immunol. Immunopathol.* **127**, 167-173.
71. Wagner, B., Wimer, C., Freer, H., Osterrieder, N. and Erb, H.N. (2011) Infection of peripheral blood mononuclear cells with neuropathogenic equine herpesvirus type-1 strain Ab4 reveals intact interferon-alpha induction and induces suppression of anti-inflammatory interleukin-10 responses in comparison to other viral strains. *Vet. Immunol. Immunopathol.* **143**, 116-124.
72. Malinowski, K., Hallquist, N.A., Helyar, L., Sherman, A.R. and Scanes, C.G. (1990) Effect of different separation protocols between mares and foals on plasma cortisol and cell-mediated immune response. *J. Equine Vet. Sci.* **10**, 363-368.
73. Adams, A.A. and Horohov, D.W. (2013) The effect of an immunomodulator (parapoxvirus ovis) on cell-mediated immunity (CMI) in abruptly weaned foals. *Vet. Immunol. Immunopathol.* **153**, 118-122.
74. Soboll-Hussey, G., Hussey, S.B., Wagner, B., Horohov, D.W., Van de Walle, G.R., Osterrieder, N., Goehring, L.S., Rao, S. and Lunn, D.P. (2011) Evaluation of immune responses following infection of ponies with an EHV-1 ORF1/2 deletion mutant. *Vet. Res.* **42**, 23-9716-42-23.
75. Goodman, L.B., Wimer, C., Dubovi, E.J., Gold, C. and Wagner, B. (2012) Immunological correlates of vaccination and infection for equine herpesvirus 1. *Clin. Vaccine Immunol.* **19**, 235-241.
76. Mizukoshi, F., Maeda, K., Hamano, M., Iwata, H., Matsumura, T., Kondo, T. and Sugiura, T. (2002) IgG antibody subclass response against equine herpesvirus type 4 in horses. *Vet. Immunol. Immunopathol.* **88**, 97-101.
77. Dowdall, S.M., Matthews, J.B., Mair, T., Murphy, D., Love, S. and Proudman, C.J. (2002) Antigen-specific IgG(T) responses in natural and experimental cyathostominae infection in horses. *Vet. Parasitol.* **106**, 225-242.
78. Mealey, R.H., Kappmeyer, L.S., Ueti, M.W., Wagner, B. and Knowles, D.P. (2012) Protective effects of passively transferred merozoite-specific antibodies against *Theileria equi* in horses with severe combined immunodeficiency. *Clin. Vaccine Immunol.* **19**, 100-104.
79. Wagner, B. (2009) IgE in horses: occurrence in health and disease. *Vet. Immunol. Immunopathol.* **132**, 21-30.
80. Lewis, M.J., Wagner, B., Irvine, R.M. and Woof, J.M. (2010) IgA in the horse: cloning of equine polymeric Ig receptor and J chain and characterization of recombinant forms of equine IgA. *Mucosal Immunol.* **3**, 610-621.
81. Perkins, G.A., Nydam, D.V., Flaminio, M.J. and Ainsworth, D.M. (2003) Serum IgM concentrations in normal, fit horses and horses with lymphoma or other medical conditions. *J. Vet. Intern. Med.* **17**, 337-342.

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