

Viral infection and allergy – What equine immune responses can tell us about disease severity and protection

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ABSTRACT

Horses have many naturally occurring diseases that mimic similar conditions in humans. The ability to conduct environmentally controlled experiments and induced disease studies in a genetically diverse host makes the horse a valuable intermediate model between mouse studies and human clinical trials. This review highlights important similarities in the immune landscape between horses and humans using current research on two equine diseases as examples. First, equine herpesvirus type 1 (EHV-1) infection initiates a series of innate inflammatory signals at its mucosal entry site in the upper respiratory tract. These inflammatory markers are highly synchronized and predictable between individuals during viral respiratory infection and ultimately lead to adaptive immune induction and protection. The timing of early inflammatory signals, followed by specific adaptive immune markers correlating with immunity and protection, allow accurate outbreak tracking and also provide a foundation for understanding the importance of local mucosal immunity during other viral respiratory infections. Second, rare peripheral blood immune cells that promote allergic inflammation can be analyzed during *Culicoides* hypersensitivity, a naturally occurring type I IgE-mediated allergic disease of horses. Rare immune cells, such as IgE-binding monocytes or basophils, can be studied repeatedly in the horse model to unravel their larger mechanistic role in inflammation during allergic and other inflammatory diseases. We conclude with a survey of all other common equine inflammatory conditions. Together, this review serves as a reference and rationale for the horse as a non-rodent model for immunological research.

1. Introduction

The immune system works as a complex symphony of protein signals and migrating cells. The interplay of the soluble immune signals and the initiation of cellular activation or regulation depends heavily on the microenvironment at local tissues. Immune responses in heterogenous species, such as horses and humans, show a range of diversity that is influenced by differences in the individual's genetic background including immune system genes (Radbruch and Isaacs, 2009). In addition, the intricate balance of an immune response is fine-tuned by the environment of the individual – including diet, exercise, geography, exposure to insects and disease vectors, vaccinations, and many other factors (Rothhammer and Quintana, 2019). While biological and medical research largely depend on mechanistic studies in small rodents leading ultimately to human clinical trials, there is an ever-increasing need for intermediate animal models that can simultaneously inform

about specific immune mechanisms and systemic clinical implications (Hein and Griebel, 2003). A common challenge in translating biological research to clinical medicine and successful treatments is moving from local, specific cell-to-cell interactions to the larger whole-body system. As mentioned above, part of this challenge exists because humans are diverse and heterogenous, therefore making the systemic immune landscape difficult to predict when therapeutic interventions have only been tested in genetically homogeneous mouse strains, each equivalent to a single human (Hein and Griebel, 2003; Mestas and Hughes, 2004; Radbruch and Isaacs, 2009).

Research in mice has revealed major insights into immunology ranging from the discovery of key principles of immunity to the detailed mechanistic understanding of immune responses during a wide spectrum of diseases (Mestas and Hughes, 2004). The availability of immunological tools and reagents and the ability to genetically modify laboratory mice are unmatched advantages for immunological research

Abbreviations: CTL, cytotoxic T-lymphocyte; *Cul*, *Culicoides*; EHM, equine herpesvirus myeloencephalopathy; EHV-1, equine herpesvirus type 1; IBH, insect bite hypersensitivity; Treg, T regulatory cells; URT, upper respiratory tract.

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using small rodent models (Hein and Griebel, 2003). However, natural disease models in other animals can fill niches of knowledge that mice cannot provide. Large animals represent a heterogeneous population with a range of individual immunological responses while still offering the ability for controlled experimental studies (Hein and Griebel, 2003). In contrast to human studies, many confounding parameters in the environment of the animals can be restrained and variables can be synchronized. Due to large blood volumes, repeated analyses of rare immune cells from the same individual are easy to achieve and longitudinal studies with repeated sampling of large volumes is possible during experimental studies in large animals (Hein and Griebel, 2003). During recurrent or chronic inflammatory diseases, the immune system undergoes cellular and immunological changes that can persist over long periods of time or can be reactivated as environmental exposure to antigens change. It is possible that studying the immune response at only one timepoint could miss important inflammatory changes occurring during chronic diseases. Longitudinal studies in large animal natural disease models can provide a comprehensive picture of the immune landscape, its plasticity, and help to inform effective treatment approaches.

In addition, large animal models can add exceptional value to the analysis of immune cells and molecules that also exist in people but have no true counterparts in rodents (Mestas and Hughes, 2004). For example, monocytes in humans and horses are characterized and distinguished based on CD14 and CD16 expression (Ziegler-Heitbrock, 2014, 2015; Karagianni et al., 2020; Larson et al., 2020). These two proteins differentiate monocytes into classical, nonclassical and inflammatory monocyte subtypes and are remarkably similar in both species. In contrast, mice do not express CD14 or CD16 on their monocytes and instead are characterized by Ly6C, CCR2, CD11c, CD43, CD62L and CX3CR1 expression, which differentiate different monocyte types, but do not directly correlate to human populations (Ziegler-Heitbrock, 2014, 2015; Williams et al., 2018; Karagianni et al., 2020). Mice also lack certain secreted immune molecules that are expressed in humans and horses (Mestas and Hughes, 2004). For example, IL-8, a chemokine produced by monocytes, plays a key function in inflammatory diseases by recruiting neutrophils and basophils to sites of inflammation and is not naturally expressed in mouse cells (Lee et al., 1995; Mestas and Hughes, 2004; Hol et al., 2010). Monocytes, as well as macrophages and dendritic cells, have recently been appreciated for their role in contributing to the micro-environment responsible for many different inflammatory conditions (Stansfield and Ingram, 2015) and the horse can be used to further explore this.

In this review, we present the horse as an intermediate animal model for the regulation of inflammation. We will highlight how horse immune models of natural and induced diseases can provide environmental controls while still maintaining the complex heterogeneity of the natural immune response. We focus on recent findings in two natural diseases of the horse to emphasize similarities between horse and human immune mechanisms and the sequence of inflammatory events leading to changes in the immune landscape at both local and systemic levels. First, we describe how local inflammation contributes to immunity against equine herpesvirus infection in the upper respiratory tract and emphasize the crucial importance of local mucosal immune responses ultimately lead to protection against the virus. Second, we explore systemic innate immune mechanisms of rare peripheral blood monocytes and basophils that contribute to allergic inflammation during *Culicoides* (*Cul*) hypersensitivity, an IgE-mediated allergy against salivary proteins from biting *Cul* midges.

2. Upper respiratory infection with equine herpesvirus type 1 (EHV-1)

Studies of EHV-1 have illustrated the sequence of local inflammatory responses that occur in the early hours and days after respiratory infection and lead to long-lasting mucosal and systemic immunity

against the virus. The early interplay of mucosal immune molecules at the viral entry site provides new insights into the orchestration of protective host immunity that likely shares similarities with viral respiratory tract infections in other species.

EHV-1 is an alphaherpesvirus of the same subfamily as the human herpes simplex viruses (HSV-1, HSV-2) and varicella-zoster virus causing cold sores, genital ulcers, and chickenpox, respectively. Alphaherpesviruses have a narrow host range, a short replication cycle, and the ability to establish life-long latency (Van de Walle et al., 2008). EHV-1 infects equids worldwide and induces clinical disease ranging from mild rhinopneumonitis, to abortion or equine herpesvirus myeloencephalopathy (EHM) (Thorsen and Little, 1975; Gilkerson et al., 1999; Patel and Heldens, 2005; Goehring et al., 2006; Lunn et al., 2009; Perkins et al., 2009). Horses often become infected at a young age and, afterwards, remain latently infected for life (Lunn et al., 2009). Reactivation of the latent virus can occur during periods of stress such as transportation of horses, contact of horses at sales events, races or shows, introducing new horses into a barn or pasture herd, and other factors that cause excitement and/or immune suppression in horses. EHV-1 outbreaks are happening year after year in the US and other countries and include EHM (Henninger et al., 2007; Lunn et al., 2009; McFadden et al., 2016; Sutton et al., 2019) and abortions (Mumford et al., 1987; Damiani et al., 2014; Oladunni et al., 2019; Stasiak et al., 2020) (<http://www.equinediseasecc.org/alerts/outbreaks>). These repeated outbreaks underline the need for vaccines or vaccination strategies that provide improved protection against EHV-1 including protection from EHM, the neurologic form of the disease. The diagnosis of EHM is typically confirmed by a positive EHV-1 PCR result in a horse with neurological signs (Allen and Breathnach, 2006; Elia et al., 2006; Perkins et al., 2008; Pusterla et al., 2008). PCR diagnostics typically include the differentiation of a single point mutation in the ORF30 gene resulting in an A2254/G2254 nucleotide polymorphism. The G2254 genotype has been associated with a higher risk of developing neurologic disease (Nugent et al., 2006; Goodman et al., 2007; Smith et al., 2012) and abortions (Smith et al., 2010). However, it should be noted that both severe disease outcomes can occur after infection with the A2254 genotype (Lunn et al., 2009). In the US, the diagnosis of EHM causes subsequent isolation of infected horses and quarantine of all horses at the outbreak premise with strict biosecurity and stop of any horse movement for 21–28 days after the positive PCR result. EHV-1/EHM outbreaks thus have substantial medical and economic impacts for the horse industry.

EHV-1 is directly transmitted either by close nose-to-nose contact of horses or indirectly by contact with fomites (Lunn et al., 2009; Perkins et al., 2009). Initially EHV-1 infects the respiratory epithelium and is quickly taken up by antigen presenting cells, which transport the virus to regional lymph nodes (Baghi and Nauwynck, 2014; Kydd et al., 1994). Next, EHV-1 spreads throughout the body via cell-associated viremia (Kydd et al., 1994; Slater et al., 1994). EHM develops during cell-associated viremia if the virus enters endothelial cells of the central nervous system. In more detail, peripheral white blood cells carrying infectious virus closely interact with vascular endothelial cells in blood vessels of the spinal cord or the pregnant uterus. The consequences at these predilection sites are vasculitis, local inflammation, thrombosis, and an infarction in the spinal cord (Edington et al., 1986, 1991; Smith et al., 1996). Cell-associated viremia is thus considered the pre-requisite for the development of abortions and EHM (Vandekerckhove et al., 2011; Kydd et al., 1994; Lunn et al., 2009). Consequently, most recent vaccine development has focused on vaccines that prevent cell-associated viremia with the goal to effectively counteract EHM. In agreement with this prediction are data showing that a reduction in the magnitude and duration of cell-associated viremia decreases the risk of developing EHM (Edington et al., 1986; Allen, 2008).

2.1. Orchestration of the mucosal immune response to EHV-1 at the viral entry site

EHV-1 infection starts locally at the upper respiratory tract (URT). The mucosal immune response against the virus is essential for the mechanistic understanding of host immunity and protection. Recently, mucosal innate immunity against EHV-1 infection has been analyzed in more detail and several immune parameters involved in the first line of defense against the virus were discovered. The initial infection of the respiratory epithelium induces a rapid inflammatory host immune response characterized by a cytokine induction at the mucosal virus entry site in EHV-1 naïve or susceptible horses (Fig. 1A). This starts with local production of type I interferon (IFN- α) which is detectable in the nasal secretion 24 h post infection (pi) and peaks in most horses at 48 h pi (Soboll-Hussey et al., 2011; Wimer et al., 2018; Schnabel et al., 2018). Secretion of mucosal IFN- α in EHV-1 infected horses closely correlates with viral shedding and the highest IFN- α concentrations occur in the nasal secretions simultaneously with the peak of EHV-1 shedding on day 2 pi (Schnabel et al., 2018). Simultaneously, chemokines (CCL2 and CCL3) and soluble CD14 (sCD14) are secreted at the nasal mucosa with maximal concentrations on days 2–3 and 4pi, respectively (Wimer et al., 2018; Schnabel et al., 2018; Perkins et al., 2019; Schnabel et al., 2019). Gene expression of CCL2 and CCL5 by epithelial cells has also been described in a nasal mucosal explant model after EHV-1 infection *in vitro* (Zhao et al., 2017), suggesting that epithelial cells may contribute to the production of these two chemokines during EHV-1 infection *in vivo*. Other cytokines and chemokines, such as IL-10, IFN- γ , CCL5 and CCL11,

are also produced during the first week of EHV-1 infection as part of the local innate immune response to the virus (Wimer et al., 2018; Schnabel et al., 2018, 2019; Perkins et al., 2019). Notably, the induction of mucosal type I interferon, chemokines and cytokines is detectable while horses are shedding EHV-1. When the viral load decreases in nasal secretion, the inflammatory markers also decline. Most of these markers are undetectable by the end of the first week of infection when live virus can no longer be isolated. The increase of inflammatory markers, in particular sCD14, IFN- α and IFN- γ , is also detectable in peripheral blood during the first few days pi. However, increases in peripheral cytokines are less pronounced and of overall lower concentrations, at least in horses that develop respiratory signs but no neurologic disease after experimental infection. Interferons are also not as consistently detectable as at the local virus entry site (Wimer et al., 2018; Schnabel et al., 2018).

2.2. Mucosal antibody responses against EHV-1

The innate mucosal immune response during the first week of EHV-1 infection initiates and orchestrates adaptive immunity against the virus. In EHV-1 naïve horses or those that are not immune and susceptible to EHV-1 infection, intranasal infection provokes the production of EHV-1-specific antibodies. Horses express seven different IgG isotypes, named IgG1 to IgG7, and have one IgM, IgD, IgE and IgA isotype each (Wagner, 2006a). Out of the seven IgG isotypes, IgG4 and IgG7 share a high homology of 96 % in their coding sequences of the IgG constant heavy chains and are most prominently distinguished by two additional amino

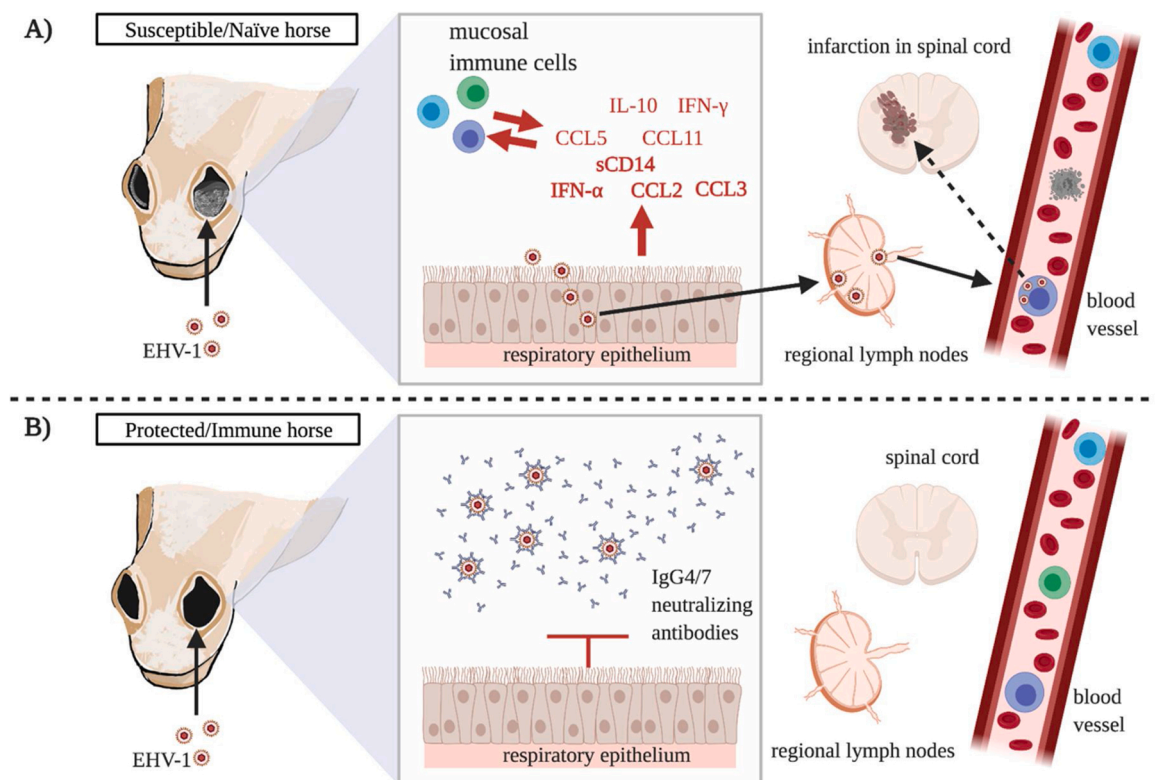


Fig. 1. EHV-1 infection of naïve or susceptible horses induces a variety of cytokines at the respiratory viral entry site while pre-existing mucosal IgG4/7 antibodies in protected or immune horses block EHV-1 entry into respiratory epithelial cells and prevent all down-stream effects of EHV-1 infection. (A) EHV-1 enters the URT and infects respiratory epithelial cells. This induces IFN- α , various chemokines and cytokines and sCD14 at the mucosa of the URT and orchestrates local innate immunity and inflammation against the virus. The cellular origin of the different chemokines and cytokines in the nasal secretion has not yet been identified. Simultaneously with the chemokine production, the EHV-1 replicates in the URT, spreads to regional lymph nodes, and subsequently spreads systemically via cell-associated viremia in the peripheral blood. During the viremic phase, EHV-1 can infect vascular endothelial cells of the central nervous system or the pregnant uterus, leading to EHM or abortions, respectively. (B) Protection from EHV-1 infection closely correlates with existing EHV-1-specific IgG4/7 antibodies. Mucosal IgG4/7 antibodies can neutralize EHV-1 at the local viral entry site and thereby prevent or significantly reduce disease, nasal virus shedding, and cell associated viremia in immune horses that are exposed to the virus. Image created with biorender.com.

acids in the hinge region of IgG7 (Wagner et al., 2004). IgG4 and IgG7 are both expressed by equine peripheral B-cells in almost equal quantities (Wagner et al., 2004). Because of their high homology, IgG4 and IgG7 can currently not be distinguished serologically and IgG4/7 has formerly been named as IgGb (Wagner, 2006a).

Local antibodies against EHV-1 are detectable in the nasal secretion at low levels as early as 2–5 days pi as a first wave of the mucosal antibody response (Wimer et al., 2018; Schnabel et al., 2018). These early local EHV-1-specific antibodies are of multiple isotypes, including different IgG isotypes and IgA, and likely represent an initial T-cell independent mucosal B-cell response to the virus at a time when systemic EHV-1 specific antibodies are still undetectable (Wagner et al., 2017; Schnabel et al., 2018; Wimer et al., 2018). By day 5–6 pi, intranasal antibody values drop for a day or two before increasing rapidly afterwards. From day 8pi on, high levels of EHV-1-specific IgG1 and IgG4/7 mucosal antibodies are present in the URT and peak around 2–3 weeks pi (Fig. 2, left graphs & gray lines). While IgG1 antibodies become almost undetectable after 2–3 months pi, EHV-1-specific IgG4/7 antibodies are maintained on the mucosal surfaces of the URT for several months (Schnabel et al., 2018) and correlate with protection from EHV-1 infection (Perkins et al., 2019; Schnabel et al., 2019). In contrast to the IgG1 and IgG4/7 response, EHV-1-specific IgA antibodies are overall low, less pronounced, decline quickly after EHV-1 infection (Schnabel et al., 2018), and do not correlate with protection (Schnabel et al., 2019).

The findings by Schnabel et al. (2018, 2019) are contradictory to a previously reported IgA dominance of equine mucosal antibodies against EHV-1 described by Breathnach et al. (2001). Both, Breathnach et al. and Schnabel et al. used the same monoclonal antibody for IgA detection in nasal secretion samples. However, Schnabel et al. used sensitive fluorescent bead-based assays based on recombinant EHV-1 antigen matrixes, while Breathnach et al. used a series of whole-virus inhibitory ELISAs for antibody detection. More recent work using sensitive detection technologies have shown that mucosal anti-EHV-1 IgA responses are of slow onset, low magnitude compared to EHV-1-specific IgG1 and IgG4/7 antibodies, and that IgA responses of EHV-1 infected horses are barely distinguishable from non-infected control horses

(Schnabel et al., 2018, 2019; and unpublished work). Thus, horses mount only weak mucosal IgA responses and mucosal immunity after EHV-1 infection is dominated by IgG4/7 antibodies.

2.3. Systemic adaptive immunity in EHV-1 naïve or susceptible horses

Infection with EHV-1 induces adaptive immunity characterized by EHV-1 specific antibodies and cell-mediated immunity in the peripheral blood (Kydd et al., 1994; Goodman et al., 2006; Kydd et al., 2006; Paillot et al., 2007; Allen, 2008; Soboll-Hussey et al., 2011; Wagner et al., 2017; Wimer et al., 2018; Schnabel et al., 2018). Serum antibodies are detectable in naturally or experimentally infected horses after recovering from disease (Goodman et al., 2006; Wagner et al., 2017; Wimer et al., 2018; Schnabel et al., 2018) and also in response to EHV vaccination with commercial vaccines (Foote et al., 2002; Goehring et al., 2010; Goodman et al., 2012; Wagner et al., 2015) or different vaccine candidates (Soboll-Hussey et al., 2011; Wimer et al., 2018; Schnabel et al., 2018). The systemic antibody response is polyclonal and directed against several EHV-1 glycoproteins (Crabb et al., 1991; Packiarajah et al., 1998; Mahmoud et al., 2013). Various studies reported the rapid induction of neutralizing antibodies by two weeks after EHV-1 infection or vaccination (Kydd et al., 2006; Goehring et al., 2010; Soboll-Hussey et al., 2011; Goodman et al., 2012). Infection of susceptible or naïve horses with EHV-1 provokes an antibody response dominated by serum IgG1 and IgG4/7 against the virus while IgG3, IgG5 and IgG6 responses are overall low (Soboll-Hussey et al., 2011; Wagner et al., 2017; Wimer et al., 2018; Schnabel et al., 2018). In addition, EHV-1 specific IgM in serum is of low magnitude and duration without a clear increase post infection even in EHV-1 naïve horses (Gibson et al., 1992; Wagner et al., 2017; Wimer et al., 2018) and EHV-1 specific IgA is not detectable in serum (Wimer et al., 2018; Schnabel et al., 2018).

Simultaneously with the mucosal antibody response discussed above, EHV-1 specific IgG1 antibodies are detectable in serum by day 8–10pi (Wagner et al., 2017; Wimer et al., 2018; Schnabel et al., 2018), peak around 2 weeks pi, and decline afterwards to reach low serum levels by 8–10 weeks pi (Fig. 2, upper right graph & gray line). The systemic IgG1 response against EHV-1 thus resembles an IgM response

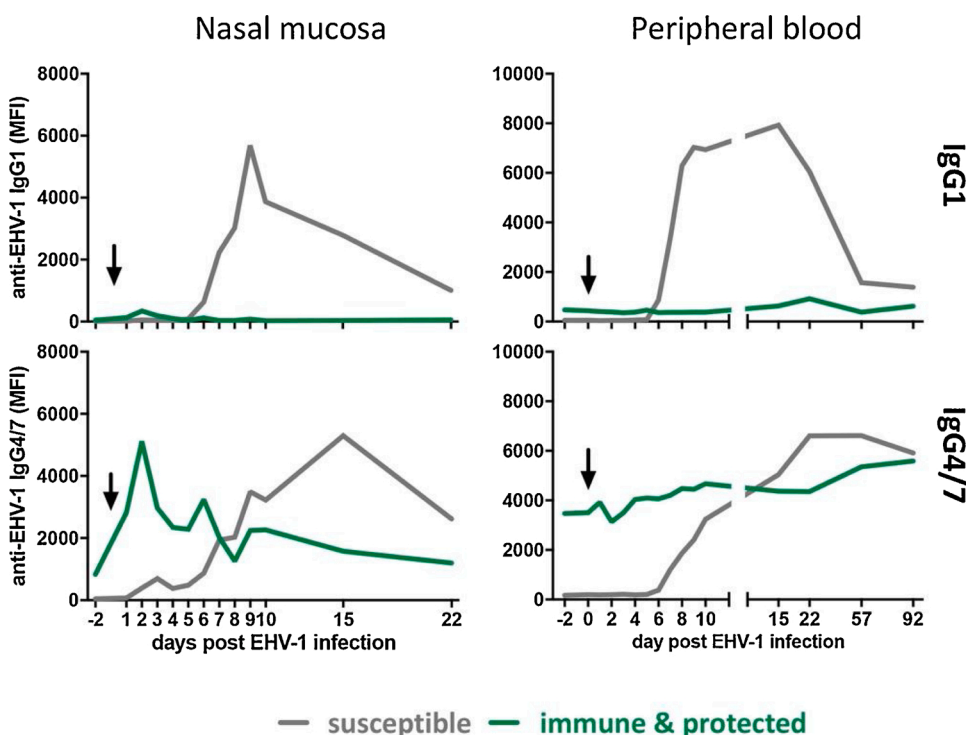


Fig. 2. Antibodies against EHV-1 at the nasal mucosa and in the peripheral blood of susceptible and protected horses. In EHV-1 naïve or susceptible horses, infection with the virus induces a strong IgG1 response by the end of the first week of infection. IgG1 antibodies are found in the nasal secretion (upper left graph) and serum of susceptible horses (upper right graph). The mucosal and systemic IgG1 responses against EHV-1 decline quickly. In protected horses, IgG1 responses are low and not upregulated after new exposure to the virus. EHV-1-specific IgG4/7 antibodies increase in susceptible horses simultaneously with IgG1 but peak later and last longer. Immune and protected horses have pre-existing IgG4/7 antibodies in their nasal secretion and serum. A new exposure to EHV-1 provokes a steep and fast increase of mucosal IgG4/7 antibodies in the protected horse, while serum IgG4/7 only increase slightly. Graphs were adapted from Perkins et al. (2019).

during primary infection. This suggests that IgG1 antibodies in horses may replace some of the early effector functions of IgM antibodies after EHV-1 infection in the naïve host. In fact, the functional characterization of the seven IgG isotypes of the horse indicates that IgG1 and IgG4/7 are the most efficient isotypes in activating the complement cascade and mediating Fc-receptor activation of other immune cells (Lewis et al., 2008).

Serum IgG4/7 antibodies against EHV-1 become detectable at the same time as IgG1 (Fig. 2, lower right graph & gray line) and increase to high levels until they plateau at around 3–4 weeks pi, then slowly decrease afterwards (Wagner et al., 2017; Wimer et al., 2018; Schnabel et al., 2018). IgG4/7 antibodies are the major IgG isotype carrying the long-term EHV-1 specific antibody response of the horse and are still detectable by 6–9 months pi, if not longer (Perkins et al., 2019; Schnabel et al., 2019).

Several inactivated and one modified-live virus vaccines are currently approved for vaccination of horses against EHV-1 alone or EHV-1 and EHV-4 combined. These vaccines provide partial protection from clinical disease, nasal shedding and viremia (Foote et al., 2002; Goodman et al., 2006; Lunn et al., 2009; Goehring et al., 2010). Overall, the antibody response after EHV vaccination resembles that after natural infection, where IgG1 and IgG4/7 isotypes dominate (Goodman et al., 2006; Goehring et al., 2010; Wagner et al., 2015, 2017). In contrast to natural EHV-1 infection, moderate to high IgG3 and IgG5 production has been reported after vaccination with some vaccines (Goodman et al., 2006; Goehring et al., 2010). Studies that directly compared neutralizing antibodies and EHV-1-specific IgG isotypes in serum after EHV-1 infection or vaccination found that IgG4/7 antibodies most closely resemble serum neutralizing antibody responses (Goehring et al., 2010; Goodman et al., 2012; Wagner et al., 2015).

EHV-1 infection of susceptible horses induces CD8⁺ cytotoxic T-lymphocyte (CTL) responses (Allen, 2008) as well as IFN- γ producing T-cells (Breathnach et al., 2005; Paillot et al., 2007; Wagner et al., 2017; Wimer et al., 2018; Schnabel et al., 2018). After infection, IFN- γ producing T-cell responses are mainly composed of cytotoxic CD8⁺ cells and fewer CD4⁺ cells (Paillot et al., 2007), but are of slow onset, low magnitude and often difficult to detect in peripheral blood (Wagner et al., 2017; Wimer et al., 2018; Schnabel et al., 2018). The main peak of cell-associated viremia typically occurs between days 4–10 after EHV-1 infection (Goodman et al., 2006; Goehring et al., 2010; Soboll-Hussey et al., 2011; Wagner et al., 2017; Wimer et al., 2018; Schnabel et al., 2018), while the onset of the peripheral T-cell response is detectable with our current methods by 3–4 weeks pi or later (Wagner et al., 2017; Wimer et al., 2018; Perkins et al., 2019). The initial CTL response is thus likely too slow for preventing the onset of EHM in EHV-1 naïve or fully susceptible horses. T-cell responses after vaccination have rarely been evaluated. One study looking at T-cell responses in pregnant mares after vaccination with an inactivated vaccine showed a slowly increasing T-helper type 1 (Th1) dominated peripheral T-cell response combined with minor activation of CD8⁺ T-cells (Wagner et al., 2015). All of these findings clearly support that EHV-1 infection induces CTL and Th1 responses. IFN- γ producing Th1 cells are required for the development of a strong IgG4/7 antibody response and the maintenance of high antibody concentrations over time. While EHV-1 specific T-cell responses may be difficult to detect in peripheral blood cells, most EHV-1 specific T-cells likely home in lymphatic tissues where they may play important roles as memory T-cells. During EHV-1 re-infection or re-activation from latency, activation of effector T-cells from existing memory is likely effective in controlling EHV-1 infected peripheral blood cells during viremia as previously described by Allen (2008).

2.4. Protection from EHV-1 infection

In the past decade, EHV-1 vaccinology has targeted the development of a new vaccine that improves T-cell immunity. Based on data published by Allen (2008), protection from EHM correlates with increased

pre-existing CTL numbers in peripheral blood. Similarly, pre-infection CTLs decrease the risk of abortion in pregnant mares (Kydd et al., 2003). The mechanistic goal of the vaccination approach targeting EHV-1 specific T-cell immunity is to control cell-associated viremia for prevention of the spread of the virus to endothelial cells. Such a vaccine does not yet exist for EHV-1.

More recent studies, characterizing both local and systemic immune responses, identified the essential role of mucosal immunity in the protection against the virus (Perkins et al., 2019; Schnabel et al., 2019). This offers a new strategy for EHV-1 vaccine development and protection from EHM outbreaks. While EHV-1 specific IgG4/7 mucosal antibodies are essential for preventing viral replication at the virus entry site, systemic EHV-1 specific IgG4/7 provide the strongest immune correlates of protection to distinguish EHV-1 susceptible and immune horses (Perkins et al., 2019; Schnabel et al., 2019). In more detail, immune horses have pre-existing IgG4/7 antibodies in their nasal secretion and high concentrations of them in serum (Fig. 2, lower graphs & green lines). After challenging an immune horse with EHV-1, intranasal IgG4/7 antibodies rapidly increase. This suggests that mucosal IgG4/7 antibodies immediately neutralize EHV-1 in the URT and prevent the virus from entering the nasal epithelium (Fig. 1B). By capturing EHV-1 right at the viral entry site, mucosal EHV-1 specific IgG4/7 antibodies can protect horses from disease, viral shedding, and cell-associated viremia after EHV-1 infection (Perkins et al., 2019; Schnabel et al., 2019). This illustrates the importance of local immunity in protecting horses against EHV-1 infection. In horses with sufficient mucosal EHV-1 specific IgG4/7 antibodies at the viral entry site, all downstream effect of infection including cell-associated viremia and endothelial cell infection do not occur or are significantly reduced. In agreement with the former statement, EHV-1 specific IgG4/7 antibodies correlate with protection from cell-associated viremia (Perkins et al., 2019; Schnabel et al., 2019) and consequently EHM.

Additional support for the role of pre-existing and rapidly increasing mucosal IgG4/7 antibodies against EHV-1 is the observation that mucosal IFN- α , chemokine and cytokine production are not detectable in immune horses after EHV-1 infection. In fact, the mucosal secretion of IFN- α and inflammatory signals is entirely missing after challenging immune horses with EHV-1 (Perkins et al., 2019; Schnabel et al., 2019). The absence of IFN- α and inflammatory markers after infection of horses with high amounts of infectious EHV-1 virus further suggests that the virus is unable to enter the respiratory epithelium of an immune horse. This conclusion is in agreement with the finding that infectious virus cannot be isolated from nasal secretions of immune horses 24 h after infection and afterwards, which implies that viral replication does not occur (Perkins et al., 2019; Schnabel et al., 2019). Thus, IFN- α and the other inflammatory signals on the respiratory mucosa should be considered danger signals and indicators of EHV-1 entering and/or replicating in the nasal epithelium. Although the individual mechanistic roles of the different inflammatory markers during replication of EHV-1 in the URT still have to be better characterized and understood, their absence in immune and protected horses is a strong indicator of the rapid inactivation of EHV-1 in the URT, most likely mediated by EHV-1 specific IgG4/7.

As mentioned earlier, IgG4/7 antibodies in serum correlate closely with serum neutralizing antibodies (Goehring et al., 2010; Goodman et al., 2012; Wagner et al., 2015) and neutralizing serum antibodies against EHV-1 correlate with protection from clinical disease (Goodman et al., 2006; Goehring et al., 2010). The same is true for EHV-1-specific IgG4/7 serum antibodies. They correlate closely with mucosal IgG4/7 and also with protection from clinical disease, nasal EHV-1 shedding and cell-associated viremia (Perkins et al., 2019; Schnabel et al., 2019). All together, these findings suggest that EHV-1 specific IgG4/7 antibodies are strong correlates of protection against EHV-1 and their existence in serum and the URT effectively prevent all disease outcomes in the individual horse.

In summary, immune responses against EHV-1 are complex. They are

initiated at the viral entry site. In the EHV-1 naïve or susceptible horse, the mucosal immune response at the URT is composed of an initial interferon, chemokine and cytokine induction and a weak T-cell independent mucosal antibody response during the first week post infection (Fig. 3). At the same time, susceptible horses experience fever, clinical respiratory signs, and shed virus via nasal secretion. Within the first week of infection, EHV-1 spreads to regional lymph nodes, cell-associated viremia starts, and EHV-1 specific adaptive immunity is induced. The risk of developing EHM is highest during viremia and EHM most commonly occurs by the end of the first week or beginning of the second week after infection. Increasing antibody responses can be detected by day 8–10 pi locally in the URT and systemically. In EHV-1 susceptible horses, the onset of adaptive immunity and highest risk of endothelial cell infection are in close proximity. While most EHV-1 susceptible horses are able to control cell-associated viremia and only develop respiratory disease, immune induction in some horses may be too slow and/or too weak to control cell-associated viremia, thereby causing the development of EHM.

In protected horses, pre-existing and rapidly increasing EHV-1 specific mucosal IgG4/7 antibodies prevent viral entry into respiratory epithelial cells and/or viral replication at the URT. This overall leads to either complete protection from disease, viral shedding and cell-associated viremia, or in a clear reduction of all of these infection outcomes including cell-associated viremia. As mentioned above, a reduction in the magnitude and duration of cell-associated viremia decreases the likelihood of EHM (Edington et al., 1986; Allen, 2008). Therefore, an EHV-1 vaccine that induces detectable and long-lasting mucosal EHV-1 specific IgG4/7 antibodies will likely be very effective in preventing EHM.

3. IgE-mediated allergy such as *Culicoides* hypersensitivity

Allergic diseases occur naturally in horses and humans in response to a wide range of targets, and both species share a similar pathomechanism of allergy (Matthews et al., 1983; van der Haegen et al., 2001; Wagner et al., 2003, 2006b, 2008, 2009, 2010, 2012; Björnsdóttir et al., 2006; Schaffartzik et al., 2012; Larson et al., 2020). Allergies, specifically IgE-mediated type I hypersensitivities, develop when there is an inappropriate inflammatory response to an external antigen or allergen. Horses, like humans, can become allergic to inhaled components of dust and mold (Ainsworth et al., 2007; Reynier et al., 2009; Bullone and Lavoie, 2020), consumed proteins in hay (Fadok, 2013; Pali-Schöll et al., 2017) and, most commonly, injected proteins from insect bites (Schaffartzik et al., 2010, 2011; van der Meide et al., 2013; Spillner et al., 2014).

The most common IgE-mediated allergy in the horse is *Culicoides* (*Cul*) hypersensitivity, also known as insect bite hypersensitivity (IBH), summer eczema, summer seasonal recurrent dermatitis, or sweet itch (Broström et al., 1987; Braverman, 1988; Larsen et al., 1988; Greiner

et al., 1990; Anderson et al., 1993; Littlewood, 1998; Steinman et al., 2003; Pilsworth and Knottenbelt, 2004; Björnsdóttir et al., 2006; Wagner, 2016). Horses who develop this allergy respond to salivary protein allergens from *Cul* midges, which reside in most regions of the world, except Iceland, New Zealand and Antarctica, during hot and humid summer months (Langner et al., 2009; Schaffartzik et al., 2010, 2011; van der Meide et al., 2013; Novotny et al., 2020). *Cul* hypersensitivity is a naturally occurring, recurrent allergy (Matthews et al., 1983; Quinn et al., 1983; Sloet van Oldruitenborgh-Oosterbaan et al., 2009). The seasonality of the disease, where allergic individuals only have clinical symptoms during months with allergen exposure, is similar to allergic rhinitis, also known as hay fever, in humans (Panzner et al., 2015).

The causal IgE-mediated pathomechanism of the disease has been confirmed through Prausnitz-Küstner passive antibody transfers to healthy, non-allergic horses followed by allergen challenge (Wagner et al., 2006b). Horses are diagnosed based on typical clinical signs in the summer (Miller et al., 2019) and causal allergens can be confirmed by intradermal allergen injections (Jose-Cunilleras et al., 2001; Morgan et al., 2007) or allergen-specific immunoglobulin measurement in serum (Morgan et al., 2007; Raza et al., 2020). Clinical signs in horses have some similarity to symptoms of human eczema and atopic dermatitis and include dermatitis, pruritis and alopecia, together disrupting the epithelial barrier and immune signature in the skin (Cvitas et al., 2020). Clinical symptom quantification was also recently standardized to allow for consistent comparison of disease between individuals (Miller et al., 2019). Variability in intradermal tests and serum IgE quantification demand more robust diagnostic methods for identifying the individual *Cul* allergens that can cause the disease. Many *Cul* allergens, primarily from *Cul obsoletus* and *Cul nubeculosus*, have been identified by cloning the different salivary proteins (Schaffartzik et al., 2010, 2011; van der Meide et al., 2012). There are similarities in these salivary proteins to allergens in bee venom that cause human bee sting allergies. For example, the enzyme hyaluronidase is both a *Culicoides* allergen Cul n 2 (Schaffartzik et al., 2011), and is also an allergen in bee, yellow jacket and hornet venom, causing anaphylaxis in humans (Spillner et al., 2014).

3.1. Icelandic horses to study the immune landscape during initial allergen exposure

While most horse breeds can develop *Cul* hypersensitivity, there is variability in disease prevalence. A few gene polymorphisms have been identified as susceptibility factors (Andersson et al., 2012; Klumplerova et al., 2013; Vychodilova et al., 2013; François et al., 2019), but small statistical differences between genetic polymorphisms in healthy and allergic horses emphasize the importance of other factors on disease onset. Specifically, prevalence is strongly associated with the time of first exposure to allergen (Björnsdóttir et al., 2006; Sommer-Locher et al., 2012; Torsteinsdóttir et al., 2018). Fewer than 10 % of horses born

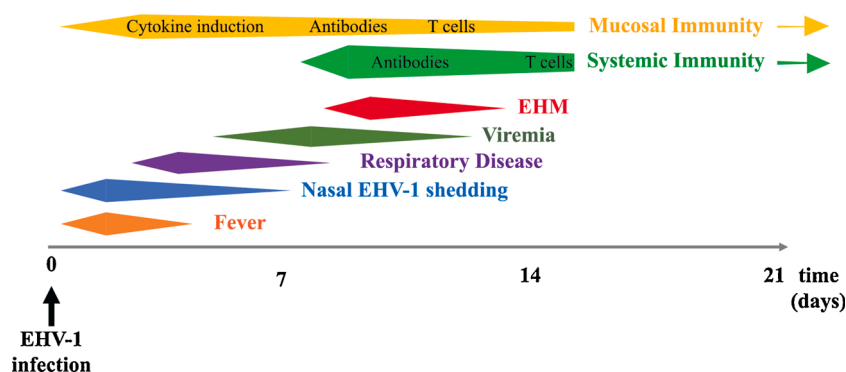


Fig. 3. Timeline of onset and duration of clinical signs, virus shedding, cell-associated viremia and immunity after EHV-1 infection of a naïve or susceptible horse.

and raised in countries with *Cul* exposure at birth develop allergy, while horses exported from Iceland to *Cul*-endemic countries develop allergy at a rate of 20–60 % (Halldórsdóttir and Larsen, 1991; Björnsdóttir et al., 2006; Raza et al., 2020). *Cul* species feeding on horses are rare and often nonexistent in Iceland and therefore Icelandic horses in Iceland are naïve to *Cul* allergens. Only upon export to a *Cul*-endemic country will the horse be exposed to allergen for the first time.

The Icelandic horse model of allergy has demonstrated that most individuals undergo silent allergen sensitization, by producing allergen-specific IgE, but only a fraction of these individuals actually develop clinical allergy upon subsequent allergen exposure (Wilson et al., 2001; Wagner et al., 2008, 2009). While both allergic and healthy horses have elevated inflammatory responses during summer months with repeated allergen exposure (Meulenbroeks et al., 2013), some authors suggested that healthy horses develop a T helper type 1 (Th1)-skewed immune response against *Culicoides* allergens (Meulenbroeks et al., 2015) and remain healthy, while allergic horses develop Th2 response and clinical signs of allergy. T cells stimulated with recombinant allergens produced elevated IL-4 mRNA transcripts in allergic horses (Meulenbroeks et al., 2015). Allergic horses have a decreased proportion of allergen-specific FOXP3 + T regulatory cells (Treg) compared to healthy controls (Hamza et al., 2012, 2013), which may in part be due to increased IL-4 that inhibits Treg development. Immune cells in skin lesions of allergic horses also comprise a pro-inflammatory environment. Skin of allergic horses have an increased frequency of CD4 + T cells, lower ratios of Treg to CD4⁺ T cells, increased IL-13 mRNA, and decreased levels of IL-10 mRNA (Heimann et al., 2011). Therefore, the early sensitization phase of allergy, before clinical symptoms develop, likely exhibits differences in immune landscape between allergic and healthy horses that may be valuable for earlier diagnosis and treatment of this condition.

3.2. Serum IgE and IgG levels in allergy

Allergies are caused by the development of allergen-specific IgE, which then induces an inflammatory response through binding to high-affinity IgE receptors on the surface of mast cells and basophils. Allergen-specific IgE measurement, along with intradermal tests, supports the diagnosis of an IgE-mediated allergy and identifies the causal allergens. While serum total IgE concentrations in horses are higher than in humans (Wagner et al., 2003, 2006b; Wilson et al., 2006), IgE still circulates at low concentrations in blood and rapidly binds to IgE receptors on cells in peripheral blood, decreasing serum IgE concentration (Qiu et al., 2020). Increased serum total IgE has been described to distinguish allergic from non-allergic horses, but these studies have reported moderate to poor sensitivity and specificity (Wilson et al., 2006; Hamza et al., 2010). In contrast, we and other groups have been unable to detect differences in serum total IgE in well-controlled groups of allergic and healthy horses, including the Icelandic horse model, living in the same region (Morgan et al., 2007; Verdon et al., 2019; Larson et al., 2020). This highlights that IgE concentrations may vary depending on the local environment, frequency of deworming and vaccination, diet, and pathogen burden; factors that vary between horse farm or region (Hamza et al., 2010). As a result, it is difficult to determine whether total IgE concentration differences are due to assay insensitivity from low concentrations, environmental variables, or truly due to clinical allergy.

Allergen-specific IgE in serum has been used to identify new *Culicoides* allergens and to determine differences in allergen-specific responses in allergic and non-allergic horses. These studies have primarily used ELISA, and more recently bead-based assays (Raza et al., 2020) or microarrays (Novotny et al., 2020). As with total IgE, allergen-specific IgE quantification has proven inconsistent in distinguishing *Cul* hypersensitive from healthy horses (Langner et al., 2008). However, many studies have identified increased *Cul*-specific IgE concentrations in allergic horses, specifically against *Cul obsoletus* whole body extract and recombinant allergens (van der Meide et al., 2012, 2014; Peeters et al.,

2013; Schurink et al., 2014; Meulenbroeks et al., 2015; Ziegler et al., 2018; François et al., 2019; Verdon et al., 2019; Wilkotek et al., 2019). Comparisons between these studies identify some potential major allergens, including cul o 1, cul o 2, cul o 3, cul o 5 and cul n 4 (van der Meide et al., 2014; Ziegler et al., 2018; François et al., 2019). However, allergen-specific IgE increases with duration and possibly severity of allergy. While horses with long-term allergy often show increased IgE values, horses with more recent onset or milder forms of allergic inflammation still have fairly low IgE values in their circulation (Raza et al., 2020).

In light of these limitations, allergen-specific IgG may provide a more robust marker earlier or predictive information on allergy development. Immunoglobulin class switching to IgE can occur sequentially in B-cells by first switching to Th2 and IL-4 induced IgG isotypes and then finally to IgE (Mosmann et al., 1986). In horses, the IgG isotypes that have been associated with Th2 responses are IgG3 and IgG5 (Perkins and Wagner, 2015). In the context of *Cul* hypersensitivity, it was recently shown that cul o 1, cul o 2 and cul o 3-specific IgG3 and IgG5 isotypes in the horse are present in circulation even before horses develop clinical allergy, and are increased in allergic horses compared to nonallergic horses before, during and after clinical signs of allergy occurred for the first time (Wagner et al., 2006b; Ziegler et al., 2018; Raza et al., 2020). This suggests that allergen-specific IgG3 and IgG5 could help diagnose the risk for allergy development early when allergen avoidance management and/or immunotherapy treatment might be most effective.

3.3. IL-4, mast cells and basophils in allergic inflammation

In both humans and horses, the main inflammatory cell mediators that respond rapidly to allergen exposure are mast cells and basophils. Mast cells are long-lived tissue resident myeloid cells that can be found in the skin of horses with *Cul* hypersensitivity (van der Haegen et al., 2001; Wagner et al., 2009), and also other local sites of allergy, such as the submucosa of the respiratory tract and the lungs (van der Haegen et al., 2005; Fadok, 2013), while basophils are short-lived circulating cells (Wagner et al., 2008) (Fig. 4). Eosinophils, another granulocyte that responds to IgE crosslinking in people, have also been identified in lesional skin of allergic horses (McKelvie et al., 1999; Benarafa et al., 2002). These three cell types contain pre-formed granules full of inflammatory mediators, such as histamine, that are immediately released after allergen contact. IgE receptor crosslinking further induces the rapid production of leukotrienes and cytokines in the cells that are exocytosed through degranulation (Marti et al., 1999; Wagner et al., 2008).

One of the cytokines produced by mast cells and basophils is IL-4 which, along with IL-13, induces IgE class switching in B cells, and has been studied in the context of different allergies and mouse models for its crucial role in the development of allergy. Equine IL-4 is produced by basophils (Wagner et al., 2010) and also T cells (Hamza et al., 2008, 2010). Interestingly, 5 day old foals have a population of peripheral IL-4+ basophils stimulated by colostrum-acquired IgE (Wagner et al., 2010). This IgE-mediated IL-4 expression early in life may be important in the development of an immune landscape biased towards or against future allergy development. Additionally, foals do not begin to produce endogenous IgE until close to one year of age (Perkins and Wagner, 2015) and maternal transfer of IgE to foals does not appear to influence future bias towards allergy (Marti et al., 2009).

3.4. Peripheral IgE-binding monocytes and other IgE+ cells

Besides basophils, two additional IgE+ cell types circulate in peripheral blood of both humans and horses: IgE-binding monocytes and CD23+ B cells (Fig. 4). All individuals, regardless of allergy, have populations of each cell type, and it is predicted that these cells engage in either pro- or anti-inflammatory mechanisms. IgE-binding monocytes express a variant of the high-affinity IgE receptor. These cells have been

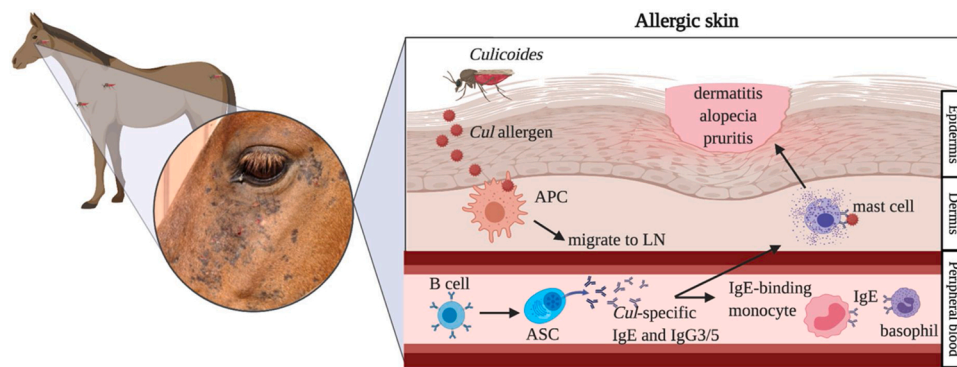


Fig. 4. Immune cell landscape during *Culicoides* hypersensitivity allergic reaction. Upon initial exposure to *Cul*, biting midges inject salivary proteins, including *Cul* allergens into the epidermis and dermis of the horse's skin. Allergens are phagocytosed by antigen presenting cells (APC), including dermal dendritic cells, which migrate to draining lymph nodes (LN) and activate allergen-specific B and T cell responses. Allergen-specific B cells class switch and ultimately differentiate into antibody secreting cells (ASC), such as plasmablasts and plasma cells, to secrete allergen-specific IgE and IgG3/5. Allergen specific IgE binds to the surface of IgE-binding monocytes and basophils in peripheral blood and mast cells in the skin. Upon subsequent allergen exposure and IgE

crosslinking, mast cells and basophils degranulate and monocytes secrete cytokines to perpetuate the allergic response, which manifests as clinical symptoms including dermatitis, alopecia and pruritis. Image created with biorender.com. Image of allergic wound on horse face adapted from Wagner (2016).

characterized in humans (Novak et al., 2001; Pyle et al., 2013), and we have recently characterized a similar population in the horse (Larson et al., 2020). In humans, IgE-binding monocytes have been shown to produce both anti-inflammatory cytokines, such as IL-10, and pro-inflammatory cytokines, such as IL-8, after IgE crosslinking (Novak et al., 2001; Pyle et al., 2013; Shin and Greer, 2015). The reactive state of IgE-binding monocytes differs in different individuals and contexts, likely based on the microenvironment of the cells. We have shown that IgE-binding monocytes in horses circulate at similar levels in all individuals and that IL-10 production from these cells is not different between allergic and healthy individuals (Larson et al., 2020). This is in contrast to human studies which showed that IL-10 secretion correlated to serum total IgE concentration and both were increased in allergic patients (Pyle et al., 2013). Recently, a role for IgE-binding monocytes to promote allergic inflammation through IL-8 production has been discovered in a horse model of natural IgE-mediated allergy. In allergic horses, IgE crosslinking provokes significantly higher IL-8 production by IgE-binding monocytes than in healthy horses living in the same environment. In contrast, IL-8 production by basophils after IgE-crosslinking is similar regardless of allergic disease (Larson et al., 2021). The findings suggest that IgE-binding monocytes from allergic individuals have increased capacity for IL-8 production and likely contribute to the recruitment of innate immune cells and inflammation during IgE-mediated allergy.

B cells also bind IgE on their surface through the low-affinity IgE-receptor, CD23. CD23+ cells remove circulating IgE to decrease IgE binding to other inflammatory cells, such as basophils. CD23+ cells can also promote the allergic response through facilitated antigen presentation (FAP). Typically, B cells bind one antigen through their B cell receptor, which can be internalized and presented to T cells through MCHII. Through CD23, B cells can internalize additional antigens that are bound by IgE on the cell surface. This process, called FAP, allows one B cell to activate multiple different T cell clones, enabling adaptive immune responses against allergens to be amplified without requiring danger signals (Galli and Tsai, 2012; Selb et al., 2017). Equine PBMC were recently phenotyped by single cell sequencing and a population of antibody secreting cells (ASC) in peripheral blood also expressed CD23, suggesting that IgE may also bind to plasmablasts as they differentiate into plasma cells (Patel et al., 2021). The regulatory and amplifying mechanisms of IgE binding to CD23+ B cells may also have implications in allergen-specific IgE secretion during allergy.

3.5. Allergen immunotherapy

Effective desensitization therapy is currently being studied and evaluated for *Cul* hypersensitivity. Treatments include vaccination with equine IL-5, the master regulator of eosinophils, to decrease eosinophil

infiltration and activation in the skin (Fettelschoss-Gabriel et al., 2018, 2019; Jonsdottir et al., 2020) and allergen immunotherapy (AIT). AIT administration techniques that have effectively induced *Cul*-specific antibody responses include oral administration (Jonsdottir et al., 2017) and intradermal or intralymphatic injections of recombinant *Cul* allergens (Barbet et al., 1990; Anderson et al., 1996; Jonsdottir et al., 2015). Treatment adjuvants for AIT have also been explored (Jonsdottir et al., 2015, 2016; Ziegler et al., 2017). Recombinant allergens have been expressed in insect cells and barley for evaluation of immune responses after immunotherapy (Jonsdottir et al., 2018). Not all immunotherapy efforts in the horse have been successful, likely due to the lack of controlled variables in these studies (Barbet et al., 1990; Ginel et al., 2014). However, these studies can guide future design and analysis of AIT in the horse.

In summary, *Cul* hypersensitivity and the study of type I IgE-mediated allergies in horses provide important insight into the sequence of immune cell migration, activation and effector function that leads to allergic inflammation and disease in some individuals, but not others. We propose that initial innate responses to *Cul* allergen by basophils, monocytes and macrophages induces a disproportionate inflammatory, IL-4 biased microenvironment that ultimately shifts subsequent responses to develop allergy. Therefore, studying the immune cell interplay during initial sensitization to allergen is critical to broaden our understanding of the mechanism of allergy development.

4. Other equine models of inflammation

Horses also experience many other naturally occurring inflammatory diseases and recent reviews and studies have specifically discussed these, often in relation to the corresponding human condition. These diseases include acute leukemia (Cooper et al., 2018), endotoxemia and septicemia (Taylor, 2015; Sheats, 2019), equine arteritis virus (Bala-suriya et al., 2013), equine hepatitis (Ramsay et al., 2015), immune-mediated muscle diseases (Durward-Akhurst and Valberg, 2018), recurrent airway obstruction (RAO) (Ferrari et al., 2018; Davis and Sheats, 2020), recurrent autoimmune uveitis (Witkowski et al., 2016), Streptococcus equi (Mallicote, 2015), inflamm-aging (McFarlane, 2016; Bullone and Lavoie, 2017), and inflammation in pregnancy (Rebordão et al., 2014; Christoffersen and Troedsson, 2017; Canisso et al., 2020). Many of these diseases, as well as equine infectious anemia, equine influenza, Rhodococcus equi, and the influence of obesity and stem cells on the immune system were also recently reviewed and compared between humans and horses (Horohov, 2015). Genetic variants in immune genes also predispose animals to many of these diseases (Tallmadge et al., 2020).

A few of these diseases have been the focus of recent projects. Equine hepatitis, caused by transmission of equine hepatitis virus (Tomlinson

et al., 2019), shares many immune parameters with human hepatitis C virus (Pfaender et al., 2017). Human hepatitis C is difficult to grow in culture, and the horse presents a unique animal model to study this disease *in vivo*. Equine recurrent uveitis is currently the only spontaneous model for recurrent autoimmune uveitis in humans, which is a T cell mediated disease against retinal proteins (Gilger, 2018). Equine T and B cells, lymphoid structures, and the immune proteome have been studied to establish this disease model (Kleinwort et al., 2016; Simeonova et al., 2016; Degroote et al., 2017; Hauck et al., 2017; Plummer, 2017). RAO, also known as equine asthma, shares many similarities with human asthma. Neutrophils are recruited to the lungs during antigen exposure in horses with RAO and are primarily responsible for disease development (Uberti and Morán, 2018). Mechanistic roles for alveolar macrophages, and cytokines such as TNF- α have also been recently explored (Montgomery et al., 2018; Wilson et al., 2020). Clinical studies of RAO/asthma treatment may serve as useful models for human asthma therapies (Leclerc, 2017; Vargas et al., 2017; Boivin et al., 2018). Characterization of additional immune cells, such as dendritic cells, macrophages, neutrophils and NK cells, is minimal and has been previously summarized (Wagner, 2016). These cells remain to be more fully studied in the context of inflammation.

5. Conclusions

Amongst many others, EHV-1 infection and *Cul* hypersensitivity represent two natural disease examples of horses that can add value to understanding the immunological landscape of inflammation, immunity and protection in heterogenous populations. We demonstrated in this review the crucial importance of local mucosal immune responses during viral respiratory infection in activating, regulating and ultimately controlling and protecting from EHV-1 infection and disease. We also emphasized the value of the controlled horse model evaluating the immune-pathogenesis of IgE-mediated allergies and in defining the roles of rare IgE-binding monocytes and basophils during allergic inflammation.

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Author contributions

EML and BW both worked on the conceptualization, the original drafting, writing, editing and review of this article.

Declaration of Competing Interest

The authors report no declarations of interest.

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