Immunological Control of Viral and Bacterial Pathogens

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Introduction
The majority of the commercial vaccines available for common equine pathogens like equine influenza virus (EIV), equine herpesvirus-1 (EHV-1) and Streptococcus equi (S. equi) are killed vaccines consisting of the inactivated pathogen or its proteins with an adjuvant, generally alum.1–3 The established measure of efficacy for these vaccines is the serum antibody (IgM and IgG) response after vaccination. Killed vaccines containing alum adjuvant produce impressive serum antibody titers by stimulating type 2 T helper lymphocyte (TH2) responses, inducing B lymphocyte activation and thus efficient antibody production. However, as reviewed by Dr. Lunn earlier, an effective immune response consists of much more than simply circulating antibody.

Generating a full and effective repertoire of adaptive immune responses to a particular pathogen is not straightforward; indeed it is only recently that the full sophistication of the immune response has become apparent.4–6 There are also significant technical difficulties in measuring other (non-antibody) aspects of immunity. Assays of the cell-mediated immune response, for example cytotoxic T lymphocyte (CTL) activity, remain technically demanding, are not suitable for batch screening, are not easily reproducible between laboratories, and are not available commercially.7–9 In contrast, antibody measurement is relatively simple, is highly suitable for batch screening, is acceptably reproducible between laboratories, and is available commercially. There can be little wonder, then, that serum antibody titers have been the standard means of assessing vaccine efficacy thus far.

However, practicing veterinarians and vaccinologists alike are well aware of the limitations of current vaccines for several of the major equine pathogens, despite the ability of these vaccines to induce efficient serum antibody responses. The aim of this paper is to explore the reasons why vaccines may fail to control viral and bacterial pathogens and thus induce protective immunity. In order to understand the reasons for this we need to consider, for each pathogen:

1. Its pathogenesis
2. The key stages in pathogenesis at which the immune system may intervene
3. The types of immune responses required to effect control at those key stages
4. The type of vaccine that would be likely to produce such immune responses

Mucosal diseases, especially respiratory diseases, present particular problems for vaccinologists be-
cause the prime requirement for protection is surface mucosal immunity rather than circulating antibody.

Certain respiratory pathogens, for example EHV-1 and \textit{S. equi}, are additionally complex because their pathogenesis involves invasion through the respiratory epithelium with potential for systemic infection.\textsuperscript{9,10} Further difficulties are presented by other respiratory pathogens, for example \textit{R. equi}, which establishes intracellular infection in macrophages and is thus shielded from effective immune surveillance.\textsuperscript{11}

We also need to remember that infection is a dynamic process by both host and pathogen. Many pathogens employ sophisticated mechanisms to impair or circumvent the host’s immune response, processes referred to as \textit{immunosuppression}. Several immunosuppressive mechanisms have been identified for EHV-1 and \textit{S. equi} and overcoming these mechanisms may represent the ultimate goal for vaccine development.\textsuperscript{12–14}

To allow us to understand the reasons why vaccines may fail to control some equine infections, we will consider the difficulties that two common respiratory pathogens, EHV-1 and \textit{S. equi}, present. The first step is to understand the pathogenesis of these infections.

**Pathogenesis of EHV-1 infections**

**Acquisition of Infection and Colonization of Respiratory Epithelium**

EHV-1 infection is acquired by inhalation of aerosols or by contact with fomites. The virus attaches to, penetrates and then replicates in epithelial cells in the nasal cavity, pharynx, trachea and bronchi\textsuperscript{15} resulting in lysis of epithelial cells and mucosal erosion (Fig. 1). The epithelial surface is thus the first site at which the immune system can neutralize EHV-1; it is also the most important site at which to maintain effective immunity: neutralization of virions here will completely prevent infection (Table 1). The physical airway defenses, consisting of the mucociliary system lining the nasal cavity, sinuses, guttural pouches, trachea and bronchi, may be able to trap virus in the mucus layer covering the respiratory epithelium and prevent virus attachment. If the horse has met EHV-1 previously the mucus layer may contain EHV-1–specific mucosal antibody directed against glycoproteins on the surface of the virion which neutralizes free virus by preventing attachment and penetration into epithelial cells. This antibody consists of IgA, secreted by submucosal B cells, and IgG, which diffuses into the epithelial mucus layer from plasma.

![Fig. 1. Colonization of respiratory epithelium by EHV-1 resulting in mucosal erosion (Fig. 1a; H&E staining, \times 40 magnification). Virus antigen expressing epithelial cells can be detected by immunoperoxidase staining (Fig. 1b). Histology images courtesy of Dr. J.A. Kydd.](image-url)

**Table 1. Key Stages in EHV-1 Pathogenesis**

<table>
<thead>
<tr>
<th>Stage</th>
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<tr>
<td>Acquisition of virus and colonization of respiratory tract epithelium*</td>
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<tr>
<td>Viraemia*</td>
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<td>Establishment of latency</td>
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<td>Infection of other organs*</td>
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</table>

* indicates stages at which an effective immune response could intervene
If a virus successfully infects respiratory epithelial cells the outcome of infection is determined by the horse's immune response. We will concentrate on the adaptive immune response because this alone is influenced by vaccination. The innate (non-specific) immune response is not influenced by vaccination and will therefore not be considered in detail in this paper. However, innate responses (consisting of complement; cytokines IL-1, IL-12, IFN-γ and TNFα, macrophages, and natural killer cells) are nonetheless extremely important in the initial limitation of EHV-1 infection before the adaptive (B and T lymphocyte) immune responses become functional.16 We should also remember that cytokines are also important in the adaptive immune response and are produced by Th lymphocytes to promote either cytotoxic (Th1) or antibody (Th2) responses. The critical role of IL-4, -5, and -6 (produced by Th2) cells in B lymphocyte stimulation is being exploited in DNA vaccination and IL-6, for example, has been included in experimental EIV DNA vaccines.

Following replication in respiratory epithelium, EHV-1 rapidly breaches the basement membrane to reach the lamina propria. Entry to the lamina propria provides the virus with opportunity for systemic dissemination via the lymphatic and circulatory systems (Fig. 2). The lamina propria is the second site at which the immune system has an opportunity to control infection. Serum neutralizing antibody probably has only a limited protective role in the lamina propria because it is restricted to neutralizing free (extracellular) virions before they have the opportunity to infect cells in the lamina propria. At this point it is important to remember that the role of antibody in the removal of EHV-1–infected cells is restricted to opsonization (aiding phagocytosis) and antibody dependent cell mediated cytotoxicity of infected cells. The protective role of antibody is therefore likely to be most effective at the epithelial surface. Once the virus has established intracellular infection, removal of virus-infected cells is achieved by cell-mediated immune responses. Virus-infected cells express virus antigens via MHC I molecules on their surface which stimulate TH1 cells and in turn CD8+ CTLs to target and lyse the infected cell. If the immune system is unable to neutralize EHV-1 in the lamina propria, it infects trafficking CD5+/CD8+ T lymphocytes and also endothelial cells, events that ultimately result in the development of a T lymphocyte-

Fig. 2. Epithelial invasion by EHV-1 and development of viraemia. Following infection of epithelial cells (Fig. 2a), EHV-1 infects endothelial cells in the lamina propria (Fig. 2b). Virus-infected mononuclear cells (mainly CD5+/CD8+) T lymphocytes subsequently appear in drainage lymph nodes (Fig. 2c) and are released into the circulation producing viraemia (virus antigens in histology sections demonstrated by immunoperoxidase staining; magnification × 40). Histology images courtesy of Dr. J.A. Kydd; cartoon courtesy of Prof. G.P. Allen.
associated viraemia. These are, of course, precisely the lymphocytes with responsibility for elimination of virus-infected cells and interference with CTL responses are one of the immunosuppressive strategies employed by EHV-1.

Viraemia

Viraemia is responsible for dissemination of virus throughout the body and the highly important sequelae of abortion, neurological disease, and chorioretinopathy. Prevention of viraemia is therefore the single most important measure of vaccine efficacy. EHV-1 viraemia is strictly cell-associated and the infected cells are mononuclear leukocytes, principally CD5+/CD8+ T lymphocytes. Immunocytochemistry studies have shown that T lymphocytes initially become infected in the lamina propria of respiratory epithelium and reach respiratory tract drainage lymph nodes within 48 h of infection (Fig. 2). Release of infected lymphocytes into the circulation via efferent lymph from drainage lymph nodes results in a T lymphocyte-associated viraemia that is detectable from as early as day 3 post infection and may persist up to day 21 post infection, although in adult horses, including pregnant mares, the duration of viraemia is usually less than 7 days. Lamina propria vascular endothelium also becomes infected and it is possible that infected endothelial cells can also contribute to viraemia via infection of circulating T lymphocytes by direct contact. The lymph node and circulation are thus the third and fourth stages at which the immune system could eliminate EHV-1 infection. However, controlling viraemia presents major problems for the immune system because 1) antibody is not effective against intracellular virus, and 2) antigen expression on infected T lymphocytes is transient, limiting the opportunity for generation of CTLs to target virus-infected lymphocytes. However, viraemic lymphocytes are only briefly targets for CTLs because the expression of viral glycoproteins on the lymphocyte surface is transient and after the early phase of viraemia, EHV-1 persists in T lymphocytes in a latent form with no virus antigen expression. Controlling viraemia clearly presents major problems for vaccine design. Killed virus vaccines result in antigen presentation via the exogenous pathway on MHC class II molecules with the induction of Th2 and antibody responses, not CTL responses.

Establishment of Latency

It seems likely that latency is established in almost all horses following infection with EHV-1. In the initial stages of nasal and conjunctival epithelial infection, EHV-1 gains access to neurons of the trigeminal nerve and reaches the trigeminal ganglion by 48 h post infection where the virus persists in a latent form. If local mucosal immunity is unable to neutralize EHV-1 at the epithelial surface and virus gains access to the trigeminal nerve receptors in the nasal epithelium, the immune system is unable to prevent the transport of virus to the trigeminal ganglion with subsequent establishment of latency in trigeminal ganglionic neurones. As mentioned above, EHV-1 also persists in a latent form in circulating CD5+/CD8+ T lymphocytes after the viremic phase of infection is terminated (Fig. 3). There is no virus replication in latently infected cells, whether these are neurons or lymphocytes, and the virus persists as genome only, presumably in the form of circular DNA as has been described for other herpesviruses. Transcriptional activity of the latent virus genome is restricted to the region antisense to the immediate early gene (ORF 64). Other genes are not transcribed and there is no viral protein translation during latency. This means that latently-infected cells do not express virus antigens and are thus antigenically silent, escaping detection, targeting and elimination by the immune system. The elimination of latent infections from the horse population is not a realistic goal for vaccination, although as discussed below, effective vaccines may be able to limit the consequences of reactivation of latent virus.

Infection of Other Organs

Viraemia is a prerequisite for the sequelae of abortion, neurological disease, and chorioretinopathies, although viraemia does not automatically induce them. Abortion is the result of ischaemia consequent to vasculitis following endothelial cell infection in uterine vasculature. This causes thrombo-ischaemic necrosis of the overlying microcotyledons and intercotyledonal stroma (Fig. 4). If these endometrial vascular lesions are widespread, the fetus may be aborted before detectable transplacental spread of virus has occurred. In other cases, disruption of the physical integrity of the uteroplacental barrier allows free virus or virus-infected cells to cross the placenta into the fetal circulation with resultant fetal infection. The mechanisms of virus transfer from infected circulating lymphocytes to vascular endothelium are unknown, although the virus antigens expressed on lymphocytes during the early stages of viraemia are glycoproteins that mediate fusion with permissive cells, for example epithelial and endothelial cells, thus providing a potential means of virus transfer.

Infection of endothelial cells, with subsequent vasculitis and ischemia, in the central nervous system (CNS) also appears to be central to the pathogenesis of neurological disease. The precise mechanism of virus transfer to the CNS is not clear but, as in the uterus, virus antigen-expressing endothelial cells can be demonstrated in the CNS by immunocytochemistry. The pathogenesis of EHV-1 induced chorioretinal lesions is less well characterized but also appears to involve endothelial cell infection with presumed ischemic injury to the choroid and retina. Although infected uterine and nervous system endothelial cells express EHV-1 antigens and are thus
subject to detection by the immune system, it seems likely that circulating antibody or CTLs would have limited opportunity to eliminate infection and prevent ischemic sequelae developing.

Reactivation

Latent virus (harbored in both circulating lymphocytes and trigeminal ganglionic neurons) is known to undergo periodic reactivation, for example in response to stressful management events (transport, handling, re-housing, and weaning) and experimentally by treatment with corticosteroids. It is therefore likely that modern, intensive management practices, especially in racing and competition horses, result in frequent reactivation of latent EHV-1 infections. Reactivation results in asymptomatic shedding of infectious virus from the respiratory tract which may result in infection of in-contact horses.

The process of reactivation of EHV-1 from neurons and circulating T lymphocytes is unknown, but must result in delivery of virions to nasopharyngeal epithelium with subsequent virus replication and shedding in nasal secretions. In neurons, reactivating virus travels from the cell body to the peripheral epithelial receptor by axonal transport. In lymphocytes, a lytic replication cycle is probably established with fusogenic viral glycoproteins on the lymphocyte surface and transfer of virus to epithelial cells (Fig. 3). The outcome of reactivation after this point is almost certainly determined by local mucosal immunity, consisting of both neutralizing antibody and CTLs. If antibody is present on the epithelial surface and antibody and CTLs are present in the lamina propria, reactivating virus is likely to be neutralized: nasal shedding of virus does not occur and reactivation is not completed. On the other hand, if mucosal immunity has not been primed (by previous infection or vaccination) or is impaired (by stress or corticosteroid treatment), reactivating virus will successfully infect epithelial cells and establish amplifying, lytic infection in these cells, thus resulting in shedding of infectious virus in nasal secretions. In these circumstances reactivation is completed. Successful completion of reactivation can also result in development of cell-associated viraemia by repeating the events following initial respiratory tract infection. This could result in dissemination of infectious virus, once again, to the uterus to produce abortion or, potentially, to the CNS to cause neurological disease. Another source of reactivated EHV-1 for causing abortion is from resident lymphocytes.
within the local environment of the pregnant endometrium which may transfer infectious virus directly to the uterine endothelium, thereby initiating the cascade of events leading to abortion. Therefore, EHV-1 abortion may occur without the prerequisite of a lytic respiratory epithelial infection or a detectable cell-associated viraemia. This may explain abortion in single mares within a group\(^2\) and also abortions that occur many weeks or months after termination of the cell-associated viraemia.\(^2\),\(^3\)

As discussed above, vaccination will not eliminate pre-existing latent EHV-1 infections. However, by stimulating and, most importantly, maintaining effective mucosal immunity, vaccines are likely to limit the frequency of nasal shedding of infectious EHV-1 following reactivation and thus limit the impact of latency in the epidemiology of this virus.

### Pathogenesis of *Streptococcus equi* Infections

Although the clinical disease and associated gross pathology caused by *S. equi* infections have been thoroughly documented, in comparison to EHV-1, the detailed events in pathogenesis are less well characterized. However, it is likely that the sequence of events in pathogenesis involves the following stages (Table 2).

#### Acquisition of Bacteria and Colonization of Respiratory Epithelium

*S. equi* is acquired directly by inhalation of ingestion of fomites from infected horses and in some cases indirectly via fomites in feed and water troughs. In contrast to other Group C streptococci found in horses, e.g., *S. zooepidemicus*, *S. equi* is not a commensal of the equine respiratory tract\(^3\) and its presence appears to be associated with either acute or chronic disease syndromes. In view of the highly contagious nature of the disease it is likely that acquisition is generally followed by successful colonization of the upper respiratory tract, particularly the nasopharynx and guttural pouches, with subsequent induction of pathology (Fig. 5).\(^3\)

The physical barrier afforded by the mucociliary apparatus and the population of the nasopharynx by commensal bacteria provides an initial obstacle to colonization. Mucosal antibody (mainly IgA but also IgG) in nasopharyngeal secretions aids in preventing colonization by binding to bacterial surface proteins (*adhesins*) required for attachment to epithelial cells and can thus provide a measure of

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**Fig. 4.** Infection of other organs by EHV-1. Abortion results from ischemia following virus infection (demonstrated by immunoperoxidase staining) of uterine endothelium causing vasculitis and thrombosis (magnification \(\times 40\)). Histology images courtesy of Dr. K.C. Smith.

<table>
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<th>Table 2. Key Stages in <em>S. equi</em> Pathogenesis</th>
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<tr>
<td>• Acquisition of bacteria and colonization of respiratory tract epithelium*</td>
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<tr>
<td>• Invasion of the lamina propria*</td>
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<tr>
<td>• Evasion of phagocytosis*</td>
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<tr>
<td>• Spread to drainage lymph nodes and the circulation*</td>
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<tr>
<td>• Abscess formation in lymph nodes and other organs*</td>
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* indicates stages at which an effective immune response could intervene

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protection against disease. As for EHV-1, the epithelium is the first site at which the immune response can eliminate S. equi infection.

If the immune system is unable to prevent adhesion of bacteria to respiratory epithelium, colonization probably progresses as follows. Adhesion to respiratory epithelium is followed by bacterial replication and production of a range of secreted toxins (for example hemolysin) which assist epithelial colonization by causing cytopathology in ciliated epithelium and impairing ciliary clearance of bacteria.

Invasion of the Lamina Propria
Following replication on the epithelium, bacteria invade the deeper epithelial layers and breach the basement membrane to penetrate the lamina propria. If bacteria are not eliminated in the lamina propria by the host immune response, they may gain access to lymphatics and reach drainage lymph nodes.

The lamina propria is thus the second site at which the immune system can eliminate bacteria. The immune response to S. equi infections differs from the immune response to EHV-1. Although initial attachment of bacteria to the epithelium may be prevented by antibody, the elimination of bacteria is achieved primarily by innate immune responses assisted by adaptive immune responses, mainly antibody. The key cells in elimination of bacteria are phagocytes (neutrophils and macrophages) assisted by B lymphocytes, not CTLs. S. equi also activates the alternative complement pathway which results in opsonization of bacteria via the binding of C3b to the bacterial surface thus facilitating phagocytosis (the process of binding of antibody or complement to bacteria resulting in enhanced phagocytosis is called opsonization). The binding of C3b to bacteria can also result in complement-mediated bacterial lysis although this is probably inefficient in Gram positive bacteria like S. equi.

Bacterial phagocytosis is greatly assisted by antibody which opsonizes bacteria and increases their susceptibility to phagocytosis. Serum antibody and mucosal antibodies are directed principally at the major bacterial antigen, the 56 kDa S. equi M-like protein SeM. The binding of antibody to S. equi surface antigens also activates the classic complement pathway, opsonizing bacteria for phagocytosis and also promoting complement-mediated bacterial lysis. In comparison to EHV-1, however, CTL responses play a minor role in bacterial elimination as S. equi is an extracellular pathogen.

Thus, in contrast to EHV-1 infections, the immune response to S. equi relies heavily on innate immunity (phagocytes and complement) augmented by adaptive immunity (antibody). Although vaccination does not influence phagocytes (in contrast to...
lymphocytes they are non-specific, do not have memory and do not exhibit anamnestic responses), stimulation of B lymphocyte responses is extremely important in recovery from infection and prevention of re-infection. The generation of mucosal antibody (from plasma cells in submucosal lymph follicles and drainage lymph nodes) and, to a lesser extent, circulating antibody, provides protection from re-infection by 1) binding to adhesins on the bacterial surface and blocking bacterial attachment to epithelium, and 2) opsonizing bacteria on the epithelium and in the lamina propria, thus greatly enhancing their removal by phagocytes. As discussed in the next section, S. equi inhibits phagocytosis and efficient removal of virulent strains of S. equi absolutely requires opsonizing antibody.

Evasion of Phagocytosis

Large numbers of phagocytes migrate to sites of S. equi infection from the circulation. S. equi is strongly chemotactic for neutrophils and the neutrophil appears to be the principal cell responsible for bacterial clearance. Neutrophils are recruited in large numbers to respiratory tract epithelium and drainage lymph nodes. Neutrophils have a short life span (less than 72 h) in tissues and therefore migrate continuously to sites of infection until infection is eliminated. To achieve bacterial phagocytosis, neutrophils firstly adhere to S. equi and then internalize the bacteria in a structure called the phagosome. Neutrophils possess surface Fc receptors and bacteria which have antibody bound to their surface and therefore adhere more efficiently to neutrophils. Complement (C3b) bound to the bacterial surface also assists adherence to neutrophils. Antibodies are particularly effective opsonins and phagocytosis is significantly enhanced in the presence of opsonizing antibody. The generation of opsonizing antibody is therefore a key requirement for S. equi vaccines. Once internalized in the phagosome, phagocytosed bacteria are killed by a variety of mechanisms which can be divided into two groups: oxidative and non-oxidative. The oxidative mechanisms kill bacteria by the release of reactive oxygen intermediates (e.g., superoxides) and reactive nitrogen intermediates (e.g., nitric oxide). The cytoplasm of neutrophils contains lysosomes which fuse with the phagosome and kill bacteria non-oxidatively by release of potently antibacterial hydrolytic enzymes (e.g., lysozyme) and peptides (e.g., azurocidin).

A key feature in pathogenesis, and one which is a central problem for vaccines, is that S. equi possesses mechanisms that allow it to resist phagocytosis and persist despite the migration of large numbers of neutrophils to the infection site. The best characterized of these is SeM, mentioned earlier as the immunodominant antigen of S. equi. SeM has potent anti-phagocytic activity, allowing bacterial survival when incubated with neutrophils in vitro. SeM exerts its anti-phagocytic action by inhibiting the deposition of complement activation products (C3b) on the bacterial surface by binding fibrinogen. The hyaluronic acid capsule of S. equi is a further mechanism which confers resistance to phagocytosis. The importance of phagocytosis evasion for S. equi virulence has been demonstrated in vitro and in field and experimental infections where bacteria deficient in SeM or hyaluronic acid capsule have reduced pathogenicity. S. equi also produces secreted protein toxins which inhibit neutrophil function. Thus far a peripheral blood mononuclear cell mitogen and a neutrophil cytokotxin have been identified.

Spread to Drainage Lymph Nodes and the Circulation

The ability of S. equi to resist phagocytosis allows bacteria to gain entry to lymphatics in the lamina propria and thus reach respiratory tract drainage lymph nodes. Bacteria remain viable and multiply despite the continuing mobilization of large numbers of neutrophils into infected lymph nodes. The persistence of viable bacteria in the node stimulates continuing activation of the complement cascade with continuing release of inflammatory mediators and chemotaxis of neutrophils into the node. This results in formation of the lymph node abscesses seen in classical strangles. Clearance of bacteria from the node requires bacterial opsonization by antibody to facilitate efficient phagocytosis and bacterial killing.

Infection of Other Organs

If the immune response is unable to restrict S. equi to respiratory tract drainage lymph nodes, bacteria may be released into the lymphatic system and into the circulation to produce a range of sequelae including metastatic abscesses (bastard strangles) in the thorax, abdomen, CNS, and myocardium.

The immune control of bacterial spread to respiratory tract drainage lymph nodes and beyond relies on efficient opsonization of bacteria by antibody with subsequent phagocytosis and elimination of bacteria by neutrophils.

How Do We Make More Effective Respiratory Vaccines?

Having considered pathogenesis carefully, the next step is to identify the components of the immune response that effective vaccines against EHV-1 and S. equi would need to generate in order to control pathogenesis. For each of these respiratory pathogens, as we follow their pathogenesis from initial infection of the respiratory epithelium into the lamina propria and beyond into drainage lymph nodes into the circulation, the opportunity for the immune system to control infection diminishes. The golden opportunity for control of infection is at the epithelial surface. Stimulation of effective mucosal immunity is therefore the primary goal of vaccines against respiratory pathogens. With present technologies the most efficient means of inducing mucosal immunity is by local (intranasal) inoculation of vaccine
into the respiratory tract. However, the vaccine must induce the appropriate humoral and cellular immune responses, which means that alternatives to inactivated vaccines (modified live, protein, or DNA vaccines) must be sought. Although modified live vaccines stimulate efficient humoral and cellular immune responses, safety is a major concern. One further consideration is that modified live vaccines that retain immunosuppressive mechanisms of the wild-type pathogen may limit the immune response generated. Protein or DNA vaccines may prove safer alternatives to modified live vaccines, but their efficacy may be less than modified live vaccines.

However, no matter how efficiently immunogenic future vaccines become, vaccination alone will not be able to control these diseases. Effective disease control will always rely on a combination of good management and vaccination. The principles of managemental control are similar for each of these pathogens and are based on:

- Isolation and testing of new arrivals to ensure they are not incubating disease or are carriers
- Segregation of high-risk animals from low-risk animals, e.g., pregnant mares from young stock
- Management of horses in small groups and avoidance of mixing groups reduction, where possible, of management stresses
- Isolation and investigation of clinical cases

In the United Kingdom, where *S. equi* vaccines are not available and only a minority of horses are vaccinated against EHV-1, these management principles have been formalized into Codes of Practice which have reduced the incidence of EHV-1 and *S. equi* infections.

Limitations of Current EHV-1 Vaccines

Ten commercial EHV-1 vaccines have been developed and used around the world, seven of which are inactivated whole virus vaccines and three which are modified live vaccines. In adult horses, at least, these vaccines do stimulate high titers of serum antibody against EHV-1. However, the impact of these vaccines on the incidence of EHV-1 has been disappointing, with EHV-1 remaining one of the most common equine pathogens. However, it would be misleading to suggest that current vaccines have been totally ineffective: the incidence of abortion in Kentucky declined by 75% over a 20-year period after the introduction of intensive vaccination program. When the required immune response to EHV-1 is considered, the shortcomings of currently available vaccines become readily apparent. Systemically administered, inactivated virus vaccines stimulate efficient circulating antibody responses but not mucosal antibody and not CTL responses. Systemically administered modified live vaccines also stimulate circulating antibody and also CTL responses but do not stimulate efficient mucosal immunity.

Requirements of an Effective EHV-1 Vaccine

An effective EHV-1 vaccine should reduce the incidence or severity of disease, contain virus spread during outbreaks, and limit virus spread following reactivation of latent infections by:

- Providing protection from initial respiratory epithelial infection
- Preventing viraemia
- Controlling reactivation of latent virus by preventing replication of reactivating virus in respiratory epithelium

To achieve these goals EHV-1 vaccines must induce and maintain effective mucosal as well as systemic immune responses. Mucosal immunity is most important since disease will not progress if virus is neutralized at the epithelium. However, if we accept that vaccination is unlikely to provide more efficient immunity than natural infection, it is important to remember that although natural infection does produce a robust protective immunity, this is transient with a duration of between 3–6 months only.

Convalescent horses with protective immunity against re-infection have the following immune responses:

- Virus neutralizing antibody in both serum and nasopharyngeal mucus
- Viral antigen driven, CD4+ T-lymphocyte proliferative activity
- Virus-specific, CD8+ cytotoxic T-lymphocyte precursors (CTLp)
- Herpesvirus reactive natural killer (NK) lymphocyte activity
- Virus specific antibody-dependent, cell-mediated cytotoxic (ADCC) activity

These provide useful guidance for desirable vaccine-generated immune responses (Table 3). The balance between the protective effects of humoral and cellular immunity is not clear, however, since no correlation with current assays has been found.

### Table 3. Immune Requirements for EHV-1 Vaccines

<table>
<thead>
<tr>
<th>Site</th>
<th>Immune Response</th>
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<tbody>
<tr>
<td>Respiratory epithelial surface</td>
<td>1. Mucosal neutralizing antibody</td>
</tr>
<tr>
<td>Epithelial lamina propria</td>
<td>1. Systemic neutralizing antibody</td>
</tr>
<tr>
<td></td>
<td>2. Cytotoxic T lymphocytes</td>
</tr>
<tr>
<td>Lymph and lymph nodes</td>
<td>1. Systemic neutralizing antibody</td>
</tr>
<tr>
<td></td>
<td>2. Cytotoxic T lymphocytes</td>
</tr>
<tr>
<td>Circulation</td>
<td>1. Systemic neutralizing antibody</td>
</tr>
<tr>
<td></td>
<td>2. Cytotoxic T lymphocytes</td>
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between serological or cellular responses and protective immunity.

Nevertheless, it is reasonable to assume that a vaccine must generate and maintain mucosal antibody, systemic antibody, and CTLs.

Future Prospects for EHV-1 Vaccines

The envelope of EHV-1 contains at least 11 glycoproteins which are the targets of the horse’s humoral, and presumably cellular, immune responses. Glycoproteins B, C, and D are the immunodominant antigens responsible for generating virus-neutralizing antibodies and are obvious targets for study as vaccine candidates.43,44 Vaccine technologies that could utilize glycoproteins B, C, and D include recombinant vector vaccines expressing these glycoproteins, protein vaccines consisting of purified glycoproteins, and DNA vaccines comprising plasmids that contain the genes for these glycoproteins. Each of these applications has received research interest, but none of the experimental approaches taken has, as yet, resulted in a commercial vaccine. Problems have included levels of protein expression in vector systems, delivery methods and preparations for protein vaccines, and a lack of information about the immunological interplay between the glycoproteins and other components of the immune response.

Five EHV-1 glycoproteins (gB, gD, gH, gL, and gK) are essential for replication of the virus while the other six (gC, gE, gG, gI, gM, and gp300) are not required for viral growth in cell culture. The deletion of non-essential glycoprotein genes decreases the virulence of EHV-1, making these genes potential candidates for modified live vaccines. The virus contains five unique genes (ORFs 1, 2, 67, 71, and 75) that have no structural homologues in any of the herpesviruses sequenced to date.45 These have also received research interest as candidates for deletion to generate modified live vaccines.

DNA vaccination may provide an alternative, effective strategy for EHV-1 and research is currently in progress to identify suitable genes to incorporate into vaccines.

While EHV-1 places stringent demands on vaccines, the virus does not appear to exhibit significant antigenic variation suggesting that the virus is, in contrast to EIV, antigenically stable and relatively homogeneous. Having reviewed the difficulties this virus presents, this final piece of information offers at least a crumb of comfort: an effective EHV-1 vaccine is likely to be effective against almost all strains of the virus and is likely to remain effective.

Requirements for an Effective S. equi Vaccine

An effective S. equi vaccine should reduce the incidence or severity of disease, reduce bacterial spread during outbreaks, and prevent the establishment of persistent infections and carrier states by:

- Stimulating respiratory mucosal neutralizing IgA antibody to block attachment of bacteria to epithelial cells
- Stimulating systemic opsonizing IgG antibody to facilitate phagocytosis
- Generating a population of long-lived memory B cells in submucosal lymph follicles and drainage lymph nodes to produce further IgA and IgG when bacteria are subsequently encountered.

These requirements are summarized in Table 4. As discussed above, systemic vaccination is unlikely to produce effective immunity against S. equi because it promotes circulating, but not mucosal, immune responses. For example, the immunodominant S. equi antigen is SeM, making it an attractive vaccine target. However, the serum antibody responses generated by systemically-administered SeM protein vaccines are not protective.

Intranasal vaccination is more likely to induce local protective immunity. Intranasally administered, modified-live bacterial vaccines induce efficient local immunity but, as for EHV-1, safety is an issue. However, a commercial modified live S. equi (Pinnacle, Fort Dodge Animal Health) has been successfully launched in the United States, but is not available in Europe. Intranasal protein or DNA vaccines are potential future alternatives, but these must induce activation of the Th2 subset of CD4+ T lymphocytes in order to promote B cell activation.

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