Immune responses to common respiratory pathogens: problems and perspectives in equine immunology

D. HANNANT
Department of Infectious Diseases, Animal Health Trust, P.O. Box 5, Newmarket, Suffolk CB8 7DW, UK.

Introduction

Like other mammals, the horse has evolved a complex combination of non-specific and specific defence systems to combat a wide variety of parasites and infectious agents. Equine ontogeny reveals that the essential features of a mature immune system are established by about 15 weeks while the foetus is maintained within its sterile environment (Perryman, McGuire and Torbeck 1980). The maturity of the equine foetal immune response has been shown by the production of antibody to antigenic challenge *in utero* with live attenuated Venezuelan equine encephalomyelitis vaccine or xenogeneic erythrocytes (Morgan, Bryans and Mock 1975; Mock, Morgan, Jochin and Lock 1978). Recent studies have shown that high levels of antibody and specific cellular immune responses can be generated to protein antigens by immunisation of the equine foetus (Hannant *et al* 1991b). The potential of mature immune responses in the equine foetus could explain the occasional finding of pathogen-specific antibody in foetal circulation or in foetuses aborted for non-infectious reasons (Whitwell, Bowen and Hannant 1991).

There is no opportunity for transfer of maternal immunoglobulins to the equine foetus pre-partum and the foal is delivered immunologically naive at term (Mock *et al* 1978) into a contaminated environment. Immediate protection from infectious organisms is usually achieved by uptake of maternally-derived immunoglobulins in the colostrum. This source of passive immunity combined with physical barriers to infection and other non-specific (innate) immunological responses is generally sufficient to allow the foal to survive and develop specific primary immunological responses during the first few weeks of life. However, passively acquired and actively achieved immunological mechanisms of the young foal are not always sufficient to prevent infectious agents becoming established in an otherwise healthy individual. The success of infectious agents in causing disease is related to the immunological status of the host and to the capacity of the invading organism to avoid immunological attack.

The purpose of this article is to summarise current knowledge about the equine immune response to infectious agents and highlight aspects that relate to protection and recovery from infection. Of necessity, much of the immunological work on equine infectious diseases has been focussed on the common respiratory pathogens. These ubiquitous agents within the horse population do not cause high mortality, but result in considerable economic loss to the performance and breeding industries and affect the recreational pursuits of horse and pony owners. Although there is considerable expertise being applied to molecular biology, genetics and antigen analyses of some important agents of equine infectious diseases such as Equid Herpesvirus, arteritis virus, influenza virus, African horse sickness, infectious anaemia and *Streptococcus equi* (Powell 1988), until recently, there has not been a similar input into fundamental immunobiology of the horse. Fortunately, recent advances in molecular and cellular biology have provided the tools for researchers to ask specific questions about the function of equine immune responses and target immunoprophylactic procedures to favour stimulation of responses that are protective.

Some components of the equine immune response

The broad field of equine immunology is reviewed in the accompanying article by Morrison (p 6), and readers are referred to this for specific explanations of the cellular immune responses and their control mechanisms. In simple terms, immunological reactions can be divided into two types: humoral and cell mediated immune responses.

Humoral immune responses

Humoral responses are mediated by antibody molecules (immunoglobulins) that are secreted by B lymphocytes and interact directly with antigens. They are formed in response to stimulation by soluble or particulate (including cell-associated) antigens that are processed in the spleen or lymph nodes. Early interest in equine immunoglobulins was stimulated about 50 years ago by the use of hyperimmune equine antisera against bacteria and bacterial toxins in the treatment and prophylaxis of human disease. Classification of equine immunoglobulins has been rather conflicting over succeeding years but current consensus supports the designation of seven antigenically distinct groups. Four groups of immunoglobulins are considered to be IgG subclasses (IgGa, IgGb, IgGc and IgG[T]) and others are represented by IgM, IgA and IgE (McGuire, Crawford and Henson 1973; Suter and Fey 1981). Biochemical properties of equine immunoglobulins were reviewed by Montgomery (1973).

Cell-mediated immune responses

Cell-mediated immunity (CMI) is characterised by induction of both cytolytic and helper T lymphocyte subsets that are restricted by class-I and class-II Major Histocompatibility Complex (MHC) gene products (Zinkernagel and Doherty 1974). Class-II MHC expression is restricted to cells with specialised antigen presenting functions or cells that have been activated by inflammatory stimuli, whereas class-I MHC molecules are expressed on most cells. Both CMI and humoral immune responses are controlled by specialised lymphoid cells and their cytokine products, such as interleukins and interferon (see Morrison p 6).

Immune responses generated by infection

The primary sites of infection and replication for respiratory pathogens are the upper respiratory tract (nasopharynx and trachea) and lower airways. This is important to understand because these are the sites where effective immune responses must function if infection and systemic spread is to be prevented. There is accumulating evidence that both humoral and cellular immune responses are stimulated in horses after natural and experimental infection with respiratory pathogens. What is not clear, however, is

the role played by each type of immune response in protection. A common finding is that a particular antibody response is generated after infection and the progressive increase in circulating antibody often coincides with a reduction in the clinical signs of disease. It is very tempting to infer that the antibody has neutralised the progressing infection and sometimes (unfortunately) it is assumed that the antibody so-generated will protect the horse from further infection. Clearly, this rationale is faulty and takes no account of the contribution of other types of antibody (eg secreted directly from the respiratory mucosal surfaces) or cellular immune responses.

It is now becoming apparent that a number of alternative routes exist in an infected cell for processing and degrading microbial antigens that affect markedly the immune responses that are generated (Weiss and Bogen 1991). For example, it is a common premise that infection by a pathogen stimulates much higher levels of immunity than exposure to inactivated antigens. The levels of cytotoxic effector and memory lymphocytes are much higher after infection exposure to influenza A viruses than after immunisation with inactivated whole virus, which is in turn more effective at stimulating immunity than virus subunit antigens (Askonas, McMichael and Webster 1982). The gradation in immunity produced is largely a function of the method by which virus antigens are processed and delivered to T lymphocytes. Details of antigen processing and the stimulation of immune effector cells can be found in Morrison's review (p 6). In brief, infection with live virus usually results in endogenous processing of viral antigens and stimulation of MHC class-I restricted cytotoxic effector cells that bear the CD8+ surface marker phenotype. The outcome of this interaction is cytolytic damage to the infected target cell (Randall and Souberbielle 1990). Exogenous processing of microbial antigens (eg by phagocytosis of virions/bacteria or partially degraded antigens) usually results in the stimulation of responding T cells, which are restricted by the MHC class-II elements of the processing cell. Lymphocytes that show class-II restriction express surface markers of the CD4+ type that are phenotypic of helper T cells (Gay *et al* 1987) and are important for local amplification of cellular and humoral immune responses and for stimulation of memory lymphocytes.

There are examples of cytotoxic effector cells that do not show the typical CD8+ surface phenotype but are CD4+ and antigen specific. These have been identified for influenza (Morrison p 6) and comprise the dominant cytotoxic cells in herpes simplex infections in other species (Schmid 1988). A third group of antigen specific cytotoxic lymphocytes has been described that functions without genetic restriction. These cells, which are characterised by the special nature of their receptor for antigen, termed gamma-delta T cell receptor (Janeway, Jones and Hayday 1988), have not been described in the horse phenotypically, but there is some evidence that they may be stimulated after equine influenza infection (Hannant and Mumford 1989).

Clearly, the initiation of immune responses at the site of infection is very complex, but some features are beginning to be understood that point the way to identifying those responses likely to be important in effective immunity. This can be described best by considering the equine immune responses to some of the common respiratory pathogens. Because of the close relationship of antibody responses at all mucosal surfaces, many of the following observations will be appropriate for studies of immune responses in the alimentary and genital tracts.

Immunity to equine influenza

Responses to infection

The typical clinical signs of equine influenza seen in infectious outbreaks (pyrexia and coughing, and in severe cases dyspnoea and anorexia) can be reproduced experimentally (Mumford, Hannant and Jessett 1990). The severity of clinical signs and levels of nasopharyngeal shedding of virus are related to the infectious virus dose. This provides a good controlled experimental system for evaluating immune responses. Early information about the duration of natural immunity or the immunity stimulated by experimental infection with equine influenza viruses suggested that high levels of antibody were required for protection (Rouse and Ditchfield 1970; Kumanomido and Akyama 1975). These findings were in accord with the studies on vaccine-induced immunity (Kumanomido and Akiyama 1975; Mumford *et al* 1983). In contrast, re-challenge experiments in ponies previously infected with influenza showed that recently exposed animals with high levels of antibody and ponies with low levels of antibody, which had been infected up to 62 weeks previously, were protected (Hannant *et al* 1988a). These observations highlight an important difference between infection-induced immunity and that provided by conventional inactivated virus vaccines where clinical immunity correlates strongly with levels of circulating antibody to the virus haemagglutinin (Wood *et al* 1983; Mumford *et al* 1983; Mumford, Wood, Folkers and Schild 1988). Similar findings have been made with human influenza where potent immunity to re-infection is established after natural or experimentally-induced primary infection (Johnson *et al* 1986), which can last for several years. In fact, Ada and Jones (1986) suggested that T cell memory to human influenza could be maintained for 10 years or more. However, when considering the duration of immunity, it is important to differentiate between the demonstration of immunological memory by *in vitro* tests and the immunologically mediated protection from second infection shown in primed individuals.

Because the primary sites for virus replication are the nasopharynx, trachea and lower airways, it is necessary to consider the contribution of local antibody and cell mediated responses to equine influenza in animals that have been immunised by previous infection. A well-developed mucosal immune system has been described in horses (Mair, Batten, Stokes and Bourne 1987a) and antibody has been detected in respiratory secretions of clinically normal horses (Mair, Stokes and Bourne 1987b). It has been difficult to ascribe functional significance to nasopharyngeal antibody in equine influenza because although it is stimulated by infection (Rouse and Ditchfield 1970; Kumanomida and Akyama 1975), there is no direct evidence to show that local antibody is protective in isolation from high background levels of serum antibody. The independence of local and circulating antibody responses to equine influenza can be inferred from their different kinetics. However, animals that were protected from infection did not show unusually high levels of nasal antibody pre-challenge, although they did show local antibody memory (Hannant, Jessett, O'Neill and Mumford 1989). It is difficult to assess the importance of locally produced antibody in sites where tissue inflammation is a feature of infection and transudation of serum antibody is likely to occur (Wagner *et al* 1987). Local antibody production in the equine nasopharynx accounts for 15 to 60 per cent of the total antibody recovered from nasal wash samples after influenza infection and, as with other species, IgA is the isotype with the longest duration (Hannant *et al* 1989).

Responses to vaccination

Systemic vaccination with inactivated whole virus vaccines is known to produce circulating antibody in horses and frequent vaccinations are required to achieve and maintain protective levels (Mumford *et al* 1983). There is some evidence for local antibody production in the equine respiratory tract after intramuscular vaccination with a virus subunit vaccine based on immune stimulating complexes (ISCOMs; Morein *et al* 1984; Hannant *et al* 1988b) but, in general, systemic vaccines stimulate local immune responses poorly. Again, this emphasises the need for more detailed studies on the contribution of local immunity to protection from equine influenza.

One possible approach is to develop vaccination schedules that favour the stimulation of mucosal antibody. An obvious approach would be to introduce the vaccine into the nasopharynx itself in an attempt to stimulate the mucosae directly. The existence of the common mucosal immune system in horses provides an alternative method for stimulating respiratory mucosal antibody independent of systemic (serum) antibody. It is well documented that the intestine responds to antigenic challenge by production of IgA-committed precursor B cells from the Peyer's patches and that these cells migrate via the efferent intestinal lymph and the thoracic duct to the blood stream (Bienenstock, McDermott, Befus and O'Neill 1978). Antigen-specific precursor cells leave the circulation and return to the intestine and other mucosal sites such as the respiratory and reproductive tracts (Pahud and Mach 1972; Widders, Stokes, David and Bourne 1985). Specific targeting of mucosal immunity should be improved by enhancing the uptake of antigen into mucosal associated lymphoid tissues (MALT) and this can be achieved by the use of a receptor mediated uptake system based on cholera toxin.

The B chain of cholera toxin is known to have a high affinity for epithelial cell membrane GMl ganglioside and this functions to introduce the toxic A chain into the cells (Holmgren 1981). Removal of the toxic A chain from the holotoxin allows the B chain to be used as a specific mucosal adjuvant, which will target antigens to MALT. This type of approach has been followed for stimulating mucosal immunity to influenza antigens in other species (Liang, Lamm and Nedrud 1988), and protection from re-challenge has been demonstrated. Mucosal stimulation with cholera toxin adjuvants also primes cellular immune responses (Clarke, Wilson, Williams and Stokes 1991).

Recent studies have shown that oral presentation of inactivated influenza virus antigens (containing approx. 100 µg virus haemagglutinin) conjugated to cholera toxin B chain is well tolerated in ponies and will stimulate new synthesis of virus specific antibody in the nasopharynx and augment pre-existing antibody levels (D. Hannant, T. O'Neill and J. A. Mumford, unpublished data). Similar results were obtained with direct application of virus toxin conjugates intranasally and studies are now in progress to measure the virus neutralising activity of locally induced antibody. Some recent work shows that the adjuvant effect of cholera toxin is not affected by the individual or strain variation of the immunised animal. Therefore, it has great potential as a vaccine adjuvant in genetically diverse animals such as horses (Hirabayashi *et al* 1991).

Stimulation of the immune responses at the first site of virus infection would appear to be a logical approach to the design of immunoprophylactic agents. The technique has wide application and theoretically, can be used with any infectious agent that invades mucosal surfaces. This may be particularly useful for agents such as *Streptococcus equi* where protection from infection seems to be related to local rather than systemic immunity (Galan and Timoney 1985a, b).

If infection with influenza is deemed to produce the best immunity, then the next best approach should be to use attenuated live vaccines that stimulate the respiratory tract and systemic immune system in much the same way. One method has been to develop attenuated live virus vaccines that show low reactogenicity but high antigenic activity in the natural host. Some experimental vaccines have been developed for use in horses based on the success of attenuated vaccines in humans and other species. Experimental strains of human influenza virus have been adapted to grow at low temperatures (eg 25°C) and used as live (intranasal) vaccines (Ghendon *et al* 1984). These vaccines (termed temperature-sensitive or cold-adapted mutants) have proved successful in stimulating long-term nasal IgA antibody (Johnson *et al* 1985) and have shown similar degrees of protection as would be induced by infection with native virus (Johnson *et al* 1985). A great advantage of these vaccines is that they are safe from adverse side effects.

Studies with live temperature sensitive mutants of influenza A/equi 1 virus (H7N7) have shown protection from homologous virus challenge four weeks after vaccination (Holmes *et al* 1988). Evidence for duration of immunity and the potential of bivalent vaccines containing both A/equi 1 and A/equi 2 (H3N8) is expected soon.

There are several groups of researchers working on other types of live attenuated equine influenza vaccines and their approaches can be broadly divided into two main areas. The first is based on the use of virus reassortants that carry the important components of the equine influenza virus envelope (haemagglutinin and neuraminidase) on the outside of a virus core derived from a non-pathogenic avian influenza virus. These 'hybrid' viruses have the capacity to attach to and infect equine cells but virus replication is much reduced. However, the reassortant virus will survive long enough to stimulate an immune response to the equine virus antigens and it is hoped that this will protect from challenge infection with native equine virus. This technique has proved valuable in animal models where significant resistance to rechallenge with native virus has been demonstrated (Murphy *et al* 1982). The safety aspects of these reassortant viruses are currently under investigation and experimental results should be available soon.

The second type of live influenza virus vaccine under investigation is based on the premise that if a virus could be identified that will produce a very strong immune response in horses (eg vaccinia), then it should be possible to construct a recombinant virus of vaccinia and equine influenza virus which would stimulate strong immunity to the latter. Vaccinia virus – equine influenza virus recombinants have been prepared and tested in horses in the USA (Dale *et al* 1988) and shown to stimulate circulating virus specific antibody. It is now necessary to assess the duration of humoral antibody and to measure cellular immune responses before challenge experiments can be undertaken.

Recent work has shown that vaccinia virus recombinants with simian immunodefficiency virus (SIV, which is a model for the human immunodefficiency virus) will induce SIV-specific CD8+ cytotoxic lymphocytes *in vivo* (Zhen *et al* 1991). Not only is this a major advance in the field of AIDS research, but it highlights the potential of vaccinia virus recombinants for stimulating virus specific cellular immunity. Such poxvirus vector systems offer great potential for stimulating both cellular and humoral immunity, and if the safety requirements for these recombinant vaccines can be achieved, we should expect them to provide good protection from challenge.

For the immediate future, ISCOM-based subunit vaccines for equine influenza will soon be available commercially. These vaccines have the property of stimulating higher levels of circulating antibody with longer duration than conventional inactivated whole virus vaccines. Therefore, once primed correctly, horses should require less frequent booster injections.

Immunity to equid herpesviruses

Responses to infection

Equid Herpesvirus (EHV) has a worldwide distribution and of the four types currently described, two are recognised as most important in the Thoroughbred racing and breeding industries (Fig 1). Type 1 (EHV-l) is associated with respiratory disease, abortion and neurological conditions that can vary from incoordination to paralysis (Allen and Bryans 1986), whereas type 4 (EHV-4) is mainly associated with respiratory disease (Patel, Edington and Mumford 1982). Horses experience first infection at a young age (Bryans 1969) and can suffer repeated exposures/infections throughout their lives. It is generally considered that immunity to infection is short-lived but the height and duration of immune responses increase after re-infection (Doll 1961). A major problem with EHV-l is that serum neutralising antibody does not protect from re-infection (Mumford *et al* 1987) and, although specific

components of cellular and local (respiratory tract) immunity develop after infection, their relation to protection is unknown. If natural infection is so ineffective, it is a major research problem to design immunoprophylactic measures to combat EHV-l.

One method is to identify those components of the equine immune response that do have a role in protection, and target them for investigation. With herpesviruses in other species, it has been shown that specific cellular immunity is generated after infection and that stimulation of cytotoxic effector cells is important for subsequent protection (Yasukawa and Zarling 1984; Zuckermann, Zsack, Mettenleiter and Ben-Porat 1990). Evidence is accumulating for the importance of MHC class-II restricted CD4+ cytotoxic lymphocytes in cellular immunity to herpesviruses in other species, in addition to the classical MHC class-I restricted CD8+ cells (Yasukawa and Zarling 1984).

Studies on cellular immunity to EHV-l and EHV-4 have been based mainly on T cell proliferation assays (Thomson and Mumford 1977; Fitzpatrick and Studdert 1984). Work on EHV-4 suggests that cytotoxic lymphocytes are induced after primary infection (Edington, Bridges, Broad and Griffiths 1988) but that type-specificity and genetic restriction of cytotoxicity is less clear after secondary infection (Bridges and Edington 1987). Because these *in vitro* cytotoxicity assays depended on exogenously processed EHV-4 antigens (see above), there is a possibility that MHC class-II restricted cytotoxic effector cells were responsible for the observed activity. Therefore, these results would be in accord with the findings in human herpesviruses, where cellular immunity is dominated by CD4+ MHC class-II restricted cytotoxic lymphocytes (Schmid 1988).

One aspect of the equine immune response to EHV-l that requires further investigation is the identification of long-term non-specific immunosuppression in horses after infection (Hannant *et al* 1991a). This immunosuppression is of long duration and does not appear to be related to the transient numerical deficit of circulating lymphocytes and neutrophils seen after EHV-l infection. The mechanism for this is unknown but it may affect the capacity of animals to respond to other infectious agents or vaccines for several weeks after an infection with EHV-l.

Although EHV-l is transmitted via the respiratory route, the virus has an endothelial cell tropism (Edington, Bridges and Patel 1986) and spreads throughout the body as a cell associated viraemia. The method of virus transfer to the foetus and the mechanism of induction of abortion is not understood. Also, the establishment of latency and its reactivation by stress (Edington, Bridges and Huckle 1985), makes studies on immune function to EHV-l very difficult to carry out.

Unfortunately, the over-riding problem hindering progress on studies of equine cellular immune responses to EHV-l is the lack of reliable assays for equine lymphocyte function because no markers are available to differentiate equine lymphocyte subsets. Recently, the First International Workshop on Equine Leucocyte Antigens was set up to coordinate the characterisation of monoclonal antibody reagents for equine lymphocytes (Kydd and Antczak p 4). By the time this article appears, antibodies will be available to identify a variety of equine lymphocyte subsets including the description of CD4 and CD8 markers. This is a very exciting time for researchers in equine immunology because it will soon be possible not only to differentiate the major types of equine lymphocytes, but to purify particular cells for detailed functional studies. For example, monoclonal antibodies to equine CD markers can be used to kill or inhibit the function of cytotoxic lymphocytes *in vitro* (O'Brien, Holmes, Duffus and Lunn 1991). We expect to see several important publications within the next few months which will answer some of the fundamental questions on equine immune responses to EHV-l.

The one area of EHV-l research that has made great progress over the last few years is the study of molecular biology. Details of these developments are beyond the scope of this paper, but they will have profound effects on developments in immunology. Briefly, the viral genes coding for most of the important virus envelope glycoproteins have been isolated and the gene products are being expressed in bacteria or as recombinant glycoproteins using vaccinia virus. Many of these studies have been carried out through international collaborations between the UK, Ireland, USA and Australia. Part of this collaboration has been to examine the equine cellular immune response to one particular EHV-l glycoprotein in great detail. A study is underway to dissect the equine T helper lymphocyte responses to very small fragments (peptides) of this glycoprotein to identify the dominant peptides/peptide combinations. Because the gene and the glycoprotein sequences are now known, it has been possible to synthesise multiple peptides that encompass all the proteins contained within the entire glycoprotein molecule. These peptides have been prepared in Kentucky and shipped to Newmarket where detailed studies on equine T lymphocyte responses are underway. Because studies in molecular biology and immunology are expensive and many researchers share the same broad objectives, the important research on EHV-l will be of a similar collaborative nature.

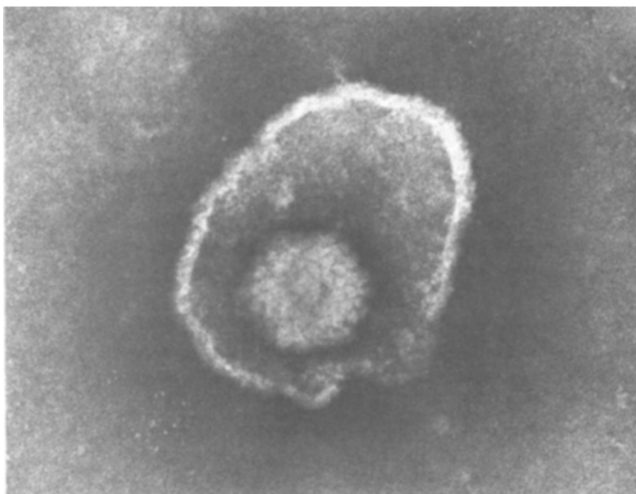

Fig 1: Electron micrograph of ENV-1. The outer virus envelope contains glycoproteins which are important in immune recognition by the horse. ENV-1 glycoproteins are contained in ISCOM vaccines and in experimental live recombinant vacccines (see text)

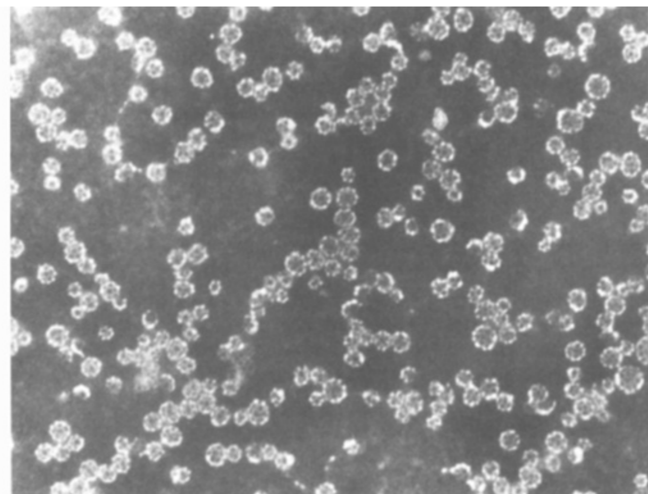

Fig 2: Experimental ENV-1 ISCOM vaccine. The major virus envelope glycoproteins are contained within the basket-like structures as revealed under high magnification (x100,000) with the electron microscope

Responses to vaccination

Although regular use of inactivated EHV-l vaccines has reduced the frequency of abortion storms in breeding establishments, there is no evidence that they will prevent infection (Mumford and Bates 1984; Bryans and Allen 1986). The circulating antibody that is produced by inactivated and live attenuated EHV-l vaccines is of rather short duration, and, as described previously, high levels of antibody do not correlate with protection. Some non-specific immunostimulants have been developed for the horse that show potential for effective treatment of clinical EHV-l infections (Leneau, Steinmeyer and Ragland 1987) but they are not appropriate for prevention of rhinopneumonitis outbreaks.

As with influenza virus, modern approaches to EHV-l immunoprophylaxis are focussed on systems that have the best potential for stimulating both cellular and humoral immunity. The ISCOM technology is being examined for EHV-l, and experimental vaccines containing defined virus glycoproteins (Cook et al 1990) are being produced. Studies in progress will evaluate the potential of EHV-l ISCOM vaccines to induce humoral and cellular immunity in horses and to protect from challenge infections (Fig 2). These and other immunological studies will benefit further from advances in molecular biology when the equine immune response to specific EHV-l gene products is better understood. Recent studies have shown that more than one of the important virus glycoproteins of EHV-l can be expressed in vaccinia virus recombinants and these have the property of inducing neutralising antibody and protection from lethal EHV-l infection in experimental animal models (Guo *et al* 1990). As with vaccinia virus-equine influenza virus recombinant vaccines, the next logical step is to evaluate their safety in horses before studying duration of immune responses and protective efficiency.

Immunity to equine arteritis virus

Little is known about the generation and duration of immunity to equine arteritis virus (EAV; Mumford 1985). Serological surveys have demonstrated the presence of EAV in most countries, but so far it has not been isolated in the UK or Ireland. Although transmission of EAV is usually via the respiratory route, it has been shown that a high percentage of stallions will shed virus in semen (Timoney 1985). Therefore, venereal transmission is important within breeding establishments. An important aspect of EAV biology is that a long-term chronic carrier state can exist in stallions that recover clinically from virus infection (Timoney *et al* 1987). The existence of the carrier state in mares is less certain.

Acute systemic disease caused by EAV infection can result in respiratory disease, paralysis and abortion (Mumford 1985). As with EHV-l, the virus spreads throughout the body via the circulatory system and replicates in endothelial cells. In animals that recover from EAV infection, high levels of serum virus-neutralising antibody are induced that have long duration. High levels of antibody correlate well with protection from respiratory but not venereal infection (McCollum *et al* 1988).

A commercially available live attenuated EAV vaccine (not licensed in UK) has induced short duration serum antibody in stallions. Although vaccine virus was never isolated from serum or urine, it was recovered sporadically from the blood and nasopharynx of vaccinated animals (Timoney, Umphenour and Collum 1988). Fukanaga *et al* (1990) have shown that multiple doses of an inactivated EAV vaccine can elicit levels of serum antibody high enough to prevent clinical disease and protect pregnant mares from abortion after challenge with virulent virus. Even though clinical disease was averted, not all horses with high antibody were protected from infection because live virus was recovered from blood samples. The value of this type of vaccine in controlling disease within stables is unclear because it is not known if the vaccinated horses showed reduced levels of virus excretion from the nasopharynx.

Molecular studies have provided the gene sequences for EAV and identified the major virus proteins (de Vries *et al* 1990; den Boon *et al* 1991) and it is hoped that these studies will be exploited to examine the equine immune response to defined virus antigens. In this way, it should be possible to target dominant immune responses by immunoprophylaxis.

Perhaps the biggest problem facing the Thoroughbred breeding and racing industries in the UK is the advance of the Single European Market legislation, which will remove mandatory serological screening of imported horses for EAV in 1992. The risk of EAV infecting British Thoroughbreds will increase, any clinical outbreak would be difficult to control, a proportion of stallions would become virus shedders and widespread vaccination programmes would be needed to contain further spread of infection. A major research effort is required to develop effective vaccines for EAV (Mumford 1985). Currently, there is only one research group working on this virus in the UK.

Immunity to equine rhinoviruses

Equine rhinoviruses are classified within the picornavirus family and are currently represented by three serotypes. Equine rhinovirus type 1 (ERV-l) is common in most horse populations and has been associated with clinical and subclinical respiratory disease (Burrows 1981). ERV-2 is rarely associated with respiratory disease, except in young animals where the pathogenesis is often compounded by secondary bacterial infection. The third serotype (ERV-3) has been described, and, although serological evidence for infection has been found, like ERV-2, it is not often associated with disease (Studdert and Gleeson 1978). There is some evidence for a long term carrier state with equine rhinoviruses, but the pathogenesis is unclear (Mumford and Thomson 1978, Steck *et al* 1978). Detections of ERV seroconversions often coincide with other virus infections such as EHV-l and EHV-4 and secondary bacterial infections (Jolly, Fu and Robinson 1986; Matsumura, Komano, Sigiura and Fu Kundga 1986; Mackintosh, Grant and Burrell 1988; Sugiura, Matsumura, Imagawa and Fukunaga 1988).

There is a high incidence of antibody to ERV in training Thoroughbreds and stud mares (Burrows 1970; Jolly *et al* 1986; Sigiura *et al* 1988) but this does not necessarily reflect recent infection events. Neutralising antibody develops very quickly after infection and persists in most animals for several months and in some cases, for years (Burrows 1970), such that more than 80 per cent of adult horses are seropositive (Jolly *et al* 1986). Long term immunity to ERV has been inferred from the persistence of neutralising antibody, but the relationships between antibody levels and protection from disease are not known.

There is no doubt that rhinoviruses are common equine pathogens, and that they are responsible for respiratory disease outbreaks in young Thoroughbreds in training. An experimental infection model needs to be developed in young horses where typical clinical signs and virus excretion can be reproduced in order to understand the function of immune responses to equine rhinoviruses.

Immunity to common respiratory bacterial infections

Many young Thoroughbreds in racing stables experience episodes of respiratory disease of uncertain cause during training. It is essential to consider the multifactorial nature of equine respiratory disease in diagnosis and treatment. However, sometimes bacterial infections are identified as the primary cause of respiratory disease in horses, and of these, the streptococci are the most important group.

Strangles

Streptococcus equi is the causative agent of this disease and in its typical form induces purulent nasal discharges, inflammation of the nasopharynx and abscessation of the adjacent lymph nodes. There are two other clinical forms of *S. equi* infection, atypical (catarrhal) and malignant (or bastard) strangles (Todd 1910). Bacterial virulence is thought to be associated with the M-like protein that stimulates formation of antibodies in affected animals (Woolcock 1974). Studies on the equine immune response to *S. equi* have shown that resistance to infection appears to be independent of the levels of bactericidal antibody in the serum (Galan and Timoney 1985a) but is related to the level of mucosal pharyngeal immune responses to the protein antigens of *S. equi*. Existing vaccines for strangles comprise killed organisms or extracts presumed to be rich in M-like protein, but they afford little protection, mainly because high levels of serum antibody are stimulated but pharyngeal mucosal immune responses are not (Galan and Timoney 1985a; Timoney and Eggers 1985).

It is clear that immunoprophylaxis of strangles in horses must be directed toward mucosal immunity. A novel approach using recombinant DNA technology has been to develop avirulent strains of *Salmonella* to carry heterologous antigens for stimulating mucosal immunity (Dougan, Hormaeche and Maskell 1987). After oral administration, *S. typhimurium* is known to invade and proliferate in the gut-associated lymphoid tissue, which leads to the development of immune responses at other mucosal sites (Bienenstock *et al* 1978). Thus, the equine common mucosal immune system can be exploited in much the same way as for inactivated virus vaccines (see above). Avirulent strains of *S. typhimurium* that contain the M protein gene of *S. equi* (Galan, Timoney and Curtis 1988) have been prepared. These bacteria express high levels of M protein in culture and experiments are in progress to study their potential as immunogens in horses. Safety aspects will be important here because *S. equi* M protein is involved in the pathogenesis of purpura haemorrhagica (Galan and Timoney 1985b). Therefore, it is important that the new vaccines stimulate protective immunity against strangles without increasing the risk of immune-complex–related diseases associated with M protein. Another problem that must be addressed is that of strain variation in *S. equi*, where a vaccine against one strain may not protect against another (Galan and Timoney 1985a). Even so, *S. equi* is one of the few equine pathogens in which mucosal immune responses are known to dominate in protection, and, now that techniques are becoming available to stimulate mucosal immunity directly, the future looks good for effective vaccines.

Streptococcus pneumoniae

Pneumococcal infection is the most common aetiological agent of community acquired pneumonia in man and *S. pneumoniae* is one of the principal pathogens (Gillespie 1989). This bacterium has been isolated from horses as a sequel to respiratory virus infection (Burrell, Mackintosh and Taylor 1986) and has been identified as a primary pathogen in young Thoroughbreds in training (Mackintosh *et al* 1988). To date, all equine isolates have been of capsule type 3 (Benson and Sweeney 1984), which in is a pathogenic strain (Austrian and Gold 1984). As with the human disease, persistent carriers of *S. pneumoniae* have been identified in horses (Mackintosh *et al* 1988).

The two major components of *S. pneumoniae* to which antibodies are produced in humans in response to infection or vaccination are the capsular polysaccharide (CPS) and the cell wall component known as C-polysaccharide. Antibody to CPS is considered essential for the survival of animals challenged with lethal doses of virulent pneumococci (Gillespie 1989). Antibodies to C-polysaccharide are found in all susceptible species and therefore are not as protective as antibodies to CPS, but they do have the advantage of being common to all 83 serotypes currently identified. Antibody production to CPS is regulated by suppressor T cells and a special type of 'amplifier' T cell that stimulates antigen-specific B cells to undergo additional rounds of proliferation, thereby increasing the amount of antibody produced (Taylor *et al* 1984; Braley-Mullen 1986).

Nothing is known about the relative importance of humoral and cellular immunity to *S. pneumoniae* in horses. However, several leads from medical research and experimental animal studies should be followed up to develop both rapid serological diagnostic tests and effective vaccines. Serology is used routinely to diagnose *S. pneumoniae* serotypes in man (Gillespie 1989) and capsular antigens may be detected in the serum and urine of patients with pneumococcal pneumonia by a serotype-specific latex agglutination test (O'Neill *et al* 1989). Serological diagnosis of *S. pneumoniae* has been reported only rarely in horses (Mackintosh *et al* 1988). Current research is directed toward development of tests suitable for serosurveys of the prevalence of *S. pneumoniae* in Thoroughbreds (J. Wood and D. Hannant, unpublished data). This information will help to identify the frequency of asymptomatic persistent carriers. High levels of antibody to CPS correlate with protection in other species (Gillespie 1989), but there is no evidence that respiratory tract antibody protects from infection with *S. pneumoniae*, although mucosal immunity is important in protection from *S. equi* (above) and other streptococcal infections (Czerkisky *et al* 1989). Therefore, it appears that the generation and duration of antibody responses to CPS offer the best first approach to targeting effective vaccines for *S. pneumoniae* in horses, particularly as the important cellular immune responses seem to be related to the regulation of antibody to CPS.

Rhodococcus equi

Rhodococcus equi was first isolated from a foal with fatal pneumonia by Magnusson (1923). The main routes of infection are respiratory, alimentary and genital, but only a few animals develop clinical disease. The alimentary route is the one by which most foals acquire subclinical infection that results in the stimulation of humoral and cellular immunity (Prescott, Ogilvie and Markham 1980; Hietala, Ardans and Sansome 1985). The respiratory route of infection is the most likely to cause the progressive pulmonary lesions associated with *R. equi* pneumonia in foals. Zink, Yager, Prescott and Fernando (1987) suggested that *R. equi* causes pneumonia in foals by persistent intracellular infection of alveolar macrophages. Pneumonia induced by *R. equi* is usually restricted to foals, but older horses that are immunocompromised or suffering concurrent infections also can be affected. Neither healthy adult horses (Yager 1987) nor foals as young as three weeks of age (Machang'u and Prescott 1991) are susceptible to experimental infection. Although this points to the possibility of immunological mechanisms protecting from infection, there are no studies that show this. Details of epidemiology, control, pathogenesis and treatment are given in the series of articles on *R. equi* pneumonia in a special issue of *Veterinary Microbiology* (Vol 14, pp 203-342) in 1987.

Several attempts have been made to protect foals from *R. equi* infection using inactivated bacteria or bacterial extracts as vaccines. Although immune responses can be demonstrated in the treated animals, there is no evidence for protection (Woolcock, Mutimer and Bowles 1987; Machang'u and Prescott 1991). Passive immunisation of newborn foals by ingestion of colostrum from immunised mares fails to protect from *R. equi* infection seven days later (Martens, Martens and Fiske p 19). Also, intravenous infusion of immune plasma to foals seven days after experimental infection does not show immunoprophylactic advantage (Chaffin, Martens, Martens and Fiske p 23). However, immune plasma does provide protection from *R. equi* if given to foals two and four days prior to experimental infection (Martens, Martens, Fiske and Hietala 1989).

These findings suggest that the immunological benefits of immune plasma are most obvious during the early stages of disease, prior to the establishment of severe pulmonary infection.

Therefore, although natural exposure and vaccination can induce specific immune responses in foals, it is difficult to gauge a protective role for immunity. Also, the contribution of local (respiratory tract) immunity to protection and recovery from *R. equi* is unknown. Clearly, there is an urgent need to identify the function of antigen specific immunological responses to this pathogen in horses before starting to develop potential vaccines.

Other common respiratory bacteria

Mackintosh *et al* (1988) isolated 31 aerobic and nine obligate anaerobic bacterial species from young Thoroughbred horses, and seven were considered to be potential pathogens, viz *S. zooepidemicus, S. pneumoniae, Bordetella bronchiseptica, Pasteurella pneumotropica, P. haemolytica, Peptostreptococcus* spp, and *Fusobacterium* spp. Episodes of bacterial infection may be preceded by clinical or subclinical infection with respiratory viruses. The resulting infection in a compromised host can, therefore, take on a more significant role in the pathogenesis of lower airways disease. For example, *S. zooepidemicus* is routinely isolated from the respiratory tracts of healthy horses, but is also associated with respiratory disease (Whitwell and Greet 1984). It is not clear if these (or other) bacterial pathogens of the equine respiratory tract can function as primary agents of disease and little is known about the importance of immune responses to them.

Predictions for the future

How will the new developments in immunology and molecular biology affect the control of infectious diseases in horses in the future? Initially it would appear that live attenuated vaccines or recombinant viral/bacterial vaccines offer the best chances for stimulating strong immune responses. These responses should be of the same levels as those achieved by natural infection, but should occur without the associated pathological damage. Although this scenario is attractive, there are a number of fundamental and applied problems that may delay the universal adoption of this type of technology. Firstly, a large amount of basic research needs to be done to identify the important antigens that should be expressed in the recombinant vaccine vectors. Moreover, the immune responses stimulated should be the ones already identified as being important in protection or recovery. We have already seen that generation of high levels of circulating antibody may have little relevance to generation of immunity. Secondly, once a desired antigen(s) has been targetted as being appropriate for expression in a recombinant vaccine system, it is important to know that the vector will be acceptable in the target species. With the Thoroughbred horse, the side reactions associated with the use of vaccinia or other pox virus vectors may limit the usefulness of these techniques. One problem is that of the possible severe reactions to secondary/booster injections. Because the immune response to vaccinia virus is very strong, it is feared that immunity to vaccinia virus within the equine population will increase and that there would be inappropriate reactions to second injections. Therefore, the recombinant vaccine would need to be good enough to stimulate long-term immunity after a single vaccination.

What would happen if a vaccinia recombinant vaccine was used to produce immunity to one equine pathogen and then it was necessary to re-vaccinate later against another equine pathogen using a vaccinia recombinant system? Does this mean that these vaccines need to be polyvalent, carry all anticipated antigens and produce life-long immunity after one inoculation? Some recent studies with experimental animals show that pre-existing immunity to vaccinia virus is detrimental to protection from influenza (Andrew 1989) and herpes simplex virus (Rooney *et al* 1988) induced by vaccinia recombinant vaccines. The former study also showed the inverse relationship between the level of pre-existing immunity to the viral vector at the time of immunisation and the level of protection induced by the recombinant vaccine.

Passive immunity (by transfer of immune serum) suppresses immune responses to vaccinia virus recombinants (Johnson, Meiten, Bender and Small 1988; Brochier *et al* 1989), but little is known about the performance of recombinant vaccines in animals under the cover of maternal antibody derived from colostrum.

Because of the rapid progress in molecular biology of equine viruses over the last few years, we will soon have the information on safety and efficacy of recombinant virus vaccines for some of the important pathogens (eg influenza and EHV-l). If safety problems can be overcome, they show great potential.

The new strain of equine influenza 2 (H3N8) recently identified in China (Webster and Yuanji 1991) is unusual in that it has possibly spread to horses directly from birds without genetic re-assortment. Because it has lost its ability to replicate in ducks, this virus probably will not be spread by migrating water birds. The molecular technology is available to analyse the important antigens of this strain and ensure their inclusion in the new vaccines under development.

The value of specific targetting of immune responses cannot be over emphasisied for those diseases for which effective immunity can be identified as a function of one component of the immune system. Soon new adjuvants and methods of presenting vaccines to the immune system are likely to be widespread in the equine world. The advent of ISCOM technology has allowed the development of subunit vaccines for use in horses against influenza and EHV-l and these have some commercial potential. An underdeveloped area is that of specific targetting of immune responses at sites where infection takes place. Although the potential for stimulation of mucosal surfaces with both inactivated and live attenuated vaccines has been identified, there are many methods to be examined. Liposome or ISCOM presentation of viral/bacterial antigens to the nasopharynx is one method that has potential in the horse.

The equine immune response to important respiratory pathogens is being dissected both in cellular and molecular terms and great progress has been made on the identification of important components with functional activity. In turn, advances in the understanding of viral/bacterial structure and function have revealed some of the important antigens likely to stimulate antibody and cellular immune responses. Collaborative studies between main study centres for equine immunology, virology, bacteriology and pathology are beginning to show important results and significant advances should be expected within the next few years.

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