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REVIEW PAPER

ADVANCES IN THE PATHOGENICITY AND IMMUNOLOGY OF EQUINE HERPESVIRUS TYPE-1

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SUMMARY

This paper reviews the pathogenicity and host immune responses to the equine herpesvirus type 1 (EHV-1), which causes rhinopneumonitis, abortion and central nervous system disorders in horses. Special emphasis was placed on the host reaction to the virus in the murine animal model, which includes the major pathogenic changes, clinical features as well as the immunity to the EHV-1 infection. Following intranasal infection of mice, EHV-1 establishes cell-associated viraemia before it spreads to the lung, where it replicates luxuriously and produces the clinical disease. The virus can be isolated from the respiratory tissues and other organs such as the spleen, liver, kidney, brain and lymph nodes. The virus may also cross the placenta to infect the foetus leading to either abortion or delivery of premature offsprings. The virus can be detected in a very low titre in the brain. Animals exposed to EHV-1 intranasally elicited low levels of antibody in the serum and respiratory secretions. Protection was observed following subsequent infection. However, protection was not observed in mice immunised via other routes. Nevertheless, several studies have revealed that antibody was not a primary factor to protect animals from EHV-1 infection. Instead, protection is correlated with the cell-mediated immune responses following strong DTH responses and infiltration of $CD4^+$ T cells in the lung. Following a series of study, it was postulated that cytotoxic T cell ($CD8^+$ T cell) activity is important in the control of both EHV-1 and EHV-4 infections. It was, therefore, concluded that $CD4^+$ and $CD8^+$ T cells were important for protection following primary and secondary EHV-1 infection. Other important immune factors include interferon (IFN), ADCC and complement-mediated antibody response. The immunosuppressive activity of EHV-1, cross-reactivity with EHV-4 and its usefulness as an immunising agent against EHV-1 were elucidated.

Keywords: equine herpesvirus, pathogenicity, immunity, murine animal model

INTRODUCTION

Equine herpesvirus-1 (EHV-1) is the causative agent of equine viral abortion (EVA) in horses. This disease is characterised by the various problems in horses ranging from respiratory diseases, neurological disorders and abortion in mares (Crabb and Studdert, 1995). Recently, a systemic form of EHV-1 infection in stallions with tropism to the genital tract has been reported (Blunden *et al.*, 1998).

Many studies have been conducted to understand the virus strategies in establishing infection. Studies of epidemiology and pathogenesis of EVA in horses have been complicated by various factors. Firstly, the disease is widespread and many horses have encountered the infection at an early age (Bryans and Allen, 1989). Secondly, once horses are infected, the virus establishes latency and remains in the host for life (Slater *et al.*, 1994). Later, the virus may be re-activated and shed (Gibson *et al.*, 1992a). Thirdly, natural immunity is normally short-lived and horses can be re-infected even in the presence of high titres of neutralising antibody (Bryans and Allen, 1989). Fourthly, EHV-1 may interact with other equine herpesviruses i.e., EHV-4 (Azmi and Field, 1995; Mohd-Azmi, 1999b) and EHV-2 (Rizvi *et al.*, 1997).

Another complication in the study of host response is the need for animals of defined EHV status. Specific-pathogen-free foals have been used, but experiments and clinical trials in such animals are limited while the number of animals available is small. Clearly, a meaningful animal laboratory model would be mostly invaluable. Several animal species have been tested to study the pathogenesis of equine viral abortion. These include rabbits (Ferrera, 1950), guinea pigs (Parnas *et al.*, 1949), day-old kitten (Hatziolos and Reagen, 1960), hamsters (Wilks and Coggins, 1977b; Stokes *et al.*, 1991a) and new-born mice (Patel and Edington, 1983).

This paper describes the usefulness of mouse model to study the pathogenicity of EHV-1 and the host immune responses to virus. Relevant findings were discussed in relation with EHV-1 infection in the natural host.

EQUINE VIRAL ABORTION IN HORSES

EHV-1 is transmitted through the respiratory route and infects susceptible horses by inhalation of aerosol. Transmission of the virus from mare to suckling foal may occur via milk and other secretions. In the early period of infection, horses usually become febrile with a biphasic hyperthermia (40.5°C to 42°C), show nasal discharge (serous and later muco-purulent), develop lymphadenopathy and in severe cases, conjunctivitis

(Gibson *et al.*, 1992a). The virus multiplies extensively in the nasopharynx, produces cell-associated viraemia, causes abortion and occasionally paresis or paralysis (Mumford and Edington, 1980). Following abortion, the virus can be isolated from many tissues including lungs, liver and spleen (Beckfriis, 1989).

The EHV-1 infection occasionally causes bronchopneumonia accompanied by exudation in trachea and lungs. This blocks small airways resulting in respiratory distress. The virus multiplies in the epithelial cells that lined the airways, producing inclusion body. Many of the affected cells desquamate into the exudate.

Viraemia is a common feature in EHV-1 infection (Mohd-Azmi, 1999a). It was shown to be cell-associated. However, viraemia has not being detected beyond the first 24 days post-infection (p.i.) (Bryans, 1969). During viraemia, the virus is distributed to many organs and may cross the placenta. The pregnant mare can be infected early during pregnancy but the incubation period between infection of the respiratory tract and abortion varies from 14 to 90 days (Doll and Bryans, 1962). Thus, more than 90% abortions were found to occur during the third trimester of pregnancy (Steinhagen, 1988). Other than abortion, the EHV-1-infected mares may deliver stillborn foals, while some are born alive but weak and soon died. Others are born healthy but become ill and die within a few days (Dixon *et al.*, 1978). Usually, there is no gross lesion found in the foetus apart from voluminous and odematous lungs. Microscopically, the infected foetal lungs were oedematous with pneumonitis and bronchiolitis containing intra-nuclear inclusion bodies. Hyaline membrane is found in new-borns that survived over 6 h after delivery (Hartley and Dixon, 1979). The virus can be detected in the endothelial cells of nasopharynx and lungs, in hepatocytes and Kupffer cells and in reticular cells of the red pulp of spleen (Jonsson *et al.*, 1989; Beckfriis, 1989).

Scrotal oedema and loss of libido have been noted in male horses with natural infection (McCarten *et al.*, 1995). An experimental study in ponies revealed that the virus localises in blood vessels of testes and epididymis while the virus can be shed venereally (Tearle *et al.*, 1996).

Establishment of the infection in nervous tissues does not directly cause paresis or paralysis (Stumbo, 1987). Unlike HSV-1, EHV-1 does not productively infect neurons. Instead, EHV-1 infects the vascular endothelium of the central nervous system leading to vasculitis and haemorrhages, and subsequent neural disease. It has been hypothesised that infection results in extensive damage to endothelial cells, which in turn is associated with thrombus formation. The lesions are located in both the spinal cord and brain (Edington *et al.*, 1986) and are believed to lead to hypoxic or ischemic necrosis of the related regions. The involvement of nervous tissues has also been demonstrated experimentally in new-born mice (Patel and Edington, 1983).

EQUINE VIRAL ABORTION IN ANIMAL MODELS

EHV-1 infection could be re-produced in kitten, rabbits and guinea pigs (Hatziolos and Reagen, 1960). In contrast, hamsters exposed to EHV-1 consistently developed severe disease (Anderson and Goodpasture, 1942). The EHV-1 strain KyA is a hamster-adapted strain derived from an EHV-1 strain that caused abortion in horses. It was found that the virus caused productive infection in hamsters (Doll *et al.*, 1953). The virus replicated in the hepatocytes and cardiac muscles and caused death within 3-4 days p.i. (Bracken and Randel, 1957; Arhelger *et al.*, 1963). Hamster model had been used to study many aspects of the infection and protection against EHV-1 (Cook *et al.*, 1990; Stokes *et al.*, 1991b). Although the virus is pathogenic in hamsters, the route of infection and the site of virus replication are different from the natural disease. In addition, the virus employed was an adapted strain (KyA) which lacks five genes namely genes 1, 2, 73, 74 and 75 (Telford *et al.*, 1992) compared to the EHV-1 strain AB4p. Thus, the KyA virus is actually different from the un-adapted strains that infect horses naturally. Therefore, the suitability of this animal model is questionable for the study of EHV-1 infection.

Mice are found to be susceptible to certain strains of EHV-1. In mice, the virus is able to establish viraemia and spreads to the lung (Nowotny *et al.*, 1987). Awan *et al.* (1990) re-examined the EHV-1 infection in mice and discovered that intranasal (i.n.) infection usually produced respiratory disease in adult mice. Following i.n. inoculation, infected mice show clinical signs including ruffled hair, dyspnoea, tachypnoea, anorexia, reduction in weight gain and hyperaemic conjuctiva from 1 day p.i. Deaths occur in severe cases; the number of deaths peaks by day 3-5 p.i. while the survivors recover after about 1 week p.i. Infectious virus can be isolated from the respiratory tissues (nasal turbinate, trachea and lungs) while a small numbers of the virus are detectable in the spleen, liver, kidney, adrenal gland, lymph nodes and buffy coat. The virus titres maximise at days 3-4 p.i. before declining. However, the virus can still be isolated from the respiratory tissue for up to 8-10 days p.i. Although the virus can be isolated from the brain, it is at a very low titre and not reproducible.

Microscopically, the virus is found to multiply in the ciliated-epithelial cells that lined the respiratory tract. The virus also multiplies in the non-ciliated epithelium of the nasal mucosa. Infection in the lung causes an intense inflammatory reaction, desquamation of epithelial cells and in combination with exudate cells, blocks the lumen of bronchi leading to respiratory distress (Azmi, 1994).

The virus appears to be capable of remaining latent in mice, which can be re-activated later by using immunosuppressive regimes (Awan *et al.*, 1991; Azmi, 1995c). Pregnant mice infected by EHV-1 are shown either to abort 3-5 days p.i. or deliver premature offspring which die within 48 h (Awan *et al.*, 1991). These observations are similar to those reported in natural infection of horses. Thus, the mouse model was found to be a reliable and relevant infection model for the study of pathogenesis and immune responses against EHV-1. To date, the mouse model has been used to study many aspects of the EHV-1 infection. These include pathogenesis and immunity (Field and Awan, 1990; Awan *et al.*, 1990; Awan *et al.*, 1991; Inazu *et al.*, 1993; Azmi and Field, 1993a; Azmi and Field, 1993b; Osterrider *et al.*, 1995; Baxi *et al.*, 1996; Bartels *et al.*, 1998) and testing of antiviral drugs (Mohd-Azmi and Field, 1997).

MECHANISMS OF IMMUNITY TO EHV-1

The immune status is dependent on the delicate balance between the host immune factors and the virus. Herpesviruses have evolved many strategies to upset this balance, enabling them to replicate and disseminate progeny. There are two primary immune factors: the antibodies and T cells. Antibodies are capable of neutralising free viruses either alone or in association with complement and evoking the ADCC of virus-infected cells. The T cells ($CD4^+$ and $CD8^+$) cannot destroy free viruses but can only recognise virus-infected cells when the processed viral antigens are associated with MHC class I (targeted by $CD8^+$ T cells) or class II (targeted by $CD4^+$ T cells) polypeptides and present on the infected cell. The recognition also needs several other immune recognition factors on T cells and target cells such as the direct interaction of CD2 and LFA-3, and the LFA-1 and ICAM-1 on T cells and target cells respectively (Springer, 1990; Clark and Ledbetter, 1994).

Antibody response to EHV-1

Mice that were primarily exposed to EHV-1 elicited an unexpectedly low level of serum antibody (Azmi, 1994). Similar phenomenon was observed in foals (Gibson *et al.*, 1992b; Tewari *et al.*, 1993). Despite low antibody response, mice that survived the intranasal inoculation with live virus were fully protected from subsequent infections. No protection was observed following immunisation via routes other than intranasal. This pointed to an important role of local specific immune responses. The IgG was found to be consistently present in the serum and respiratory secretion following primary exposure to EHV-1(Azmi, 1995b). Local IgG, supplied by the draining lymph nodes and bronchial-associated lymphoid tissues, was present at low levels (Bender *et al.*, 1990). Without the IgA, the IgG provided protection in the upper and lower respiratory tracts against an intranasal infection. This observation has been reported in other respiratory diseases (Cocker *et al.*, 1986; Chen and Quinnan, 1989). The low levels of IgG in lung could be sustained for up to 1-2 years p.i.

Although IgG and IgM were detected in mouse serum following primary and subsequent exposure to EHV-1, the IgA was not detected. The IgG produced was of isotypes IgG1, IgG2a and IgG2b. The same isotypes of antibodies were found in murine respiratory secretion in a similar pattern to that of serum.

The nature of antigens and route of inoculation have been proposed to markedly affect the pattern of immune responses to viruses such as the bovine respiratory syncytial virus (Kimman *et al.*, 1989). This could also be applied to the case of non-protective immunisation of mice following intravenous (i.v.) or intramuscular (i.m.) routes with live or heat-inactivated EHV-1 (Azmi, 1995b). An active virus replication in one or more organs is required for the stimulation of protective immune response. Protection from the subsequent i.n. challenge is likely to be conferred by local immune responses in the respiratory tract, either humoral or cell-mediated (Anderson *et al.*, 1990).

Despite relatively low antibody response, mice given a primary i.n. inoculation of live virus were protected from re-infection. In contrast, higher antibody levels were noted when mice were inoculated i.n. with inactivated virus, or inoculated via other routes (i.v. or i.m.) with either live or inactivated virus. These antibodies, however, were not protective against subsequent EHV-1 challenge (Azmi, 1994). A similar observation was noted in foals vaccinated with inactivated EHV-1, which became susceptible to EHV-1 as early as 3-5 months following vaccination (Fu *et al.*, 1986). Thus, inactivated vaccine was unsatisfactory for primary immunisation and appeared to be antigenically "weak" (Burki *et al.*, 1991).

Similar observations have also been reported for other herpesvirus. For example, vaccination of mice with inactivated pseudorabies virus (PrV) vaccine induced good serological response, but produced poor protection against challenge (Ali, 1999). This, in part,

was probably due to the destruction of immunodominant proteins, which are required intact to elicit protective immune responses. Duque *et al.* (1989) reported that 80-97% of the normal epitopes of BHV-1 (gI, gIII and gIV) are lost upon formalin inactivation. It appeared that protection against EHV-1 infection did not correlate with the level of antibody (Azmi, 1994; Azmi, 1995b; Azmi, 1995d).

Cell-mediated immunity to EHV-1

Since the weight of evidence obtained from several studies was not in favour of antibody (local or systemic) as a primary factor for protection to EHV-1 infection, an alternative explanation was required. Any hypothesis must take into account the fact that, in mice, the clearance of EHV-1 from the site of infection occurs by about 1week p.i. following primary i.n. inoculation. As described above, one alternative explanation would be the possibility of local immune responses (cell and/or antibody mediated). Cells that may be involved in the protection and recovery from respiratory infection should be situated close to the respiratory tract itself (Yap and Ada, 1978; Yap *et al.*, 1979), and may be able to infiltrate effectively the infected tissues during infection. In mice infected i.n. with EHV-1, many cells were observed to infiltrate the lung parenchyma during the first week p.i. The cellular infiltration may be under the control of viral specific T_H cell response (Sanderson *et al.*, 1992). Heavy cellular infiltration following an acute viral infection, however, can cause damage to the respiratory tissues (Curtis *et al.*, 1990).

Functionally active lymphocytes in the lung can mediate the relevant local immune responses involving T and B cells (Holt *et al.*, 1988; Abraham *et al.*, 1990). Other cells such as NK cells are capable of lysing virus-infected cells by means of ADCC, as described in the murine influenza virus model (Stein-Streileid *et al.*, 1983). The NK-activity in the lung was reported to be elevated earlier than those observed in spleen and the local production of cytokines such as IFN may be required to enhance the NK function. Cytokines such as IFN and TNF can also inhibit virus replication directly (Altinkilic and Brandner, 1988; Vacheron *et al.*, 1990).

Protection against EHV-1 infection in mice was found to correlate with the cell-mediated immune responses as evidenced by the strong DTH responses following i.n. inoculation (Azmi and Field, 1993a). The response persisted and could be detected even at 2 months p.i, and was further elevated following secondary virus inoculation. Monoclonal antibodies specific to certain T cell subsets were used to determine the relative role of lymphocyte subsets in DTH. It was found that the subset of T_{H1} cells was involved (Azmi and Field, 1993a) as demonstrated for HSV-1 (Nash *et al.*, 1980). Nevertheless, DTH responses were also known to be mediated by other alternative T cells such as $CD4^+$ CD^+ T cells (Askenase *et al.*, 1989; Herzog *et al.*, 1989). In addition to DTH, adoptive transfer of immune splenocytes was shown to protect recipient mice from EHV-1 infection (Azmi and Field, 1993a). In this case, however, donors were required to be immunised with live virus i.n. to stimulate protective immune cells. The transfer of protection through adoptive transfer of splenocytes had also been demonstrated in hamsters infected with EHV-1 (Daliri *et al.*, 1991) and in mice infected with alternative viruses such as HSV-1 (Sethi *et al.*, 1983), RSV (Cannon *et al.*, 1987) and influenza virus (Yap *et al.*, 1978). Based on these observations and together with evidences from other viral infections (Hom *et al.*, 1991; Koszinowski *et al.*, 1991), it appears that protection to EHV-1 infection is mediated mainly by immune cells (especially T cells).

Experiments using monoclonal antibodies to produce T cell-depletion (Azmi, 1994) found that $CD4^+$ T cells mediated cellular infiltration in lung while both $CD4^+$ and $CD8^+$ T cells were important in mediating protection in a primary and perhaps secondary EHV-1 infection. The successful transfer of protection depended on both cell populations. However, some other accessory cells may be required to provide help for the effector functions of certain T cells as suggested by Stohlman (1991). Splenic T cells were less protective in the absence of $CD4^+$ T_H cells. This was probably due to the requirement of recruitment of functional effector Tc cells. This requires the presence of T_H cell, which are responsible for the production of essential cytokines for differentiation of Tc cells and B cells. For example, the $CD4^+$ T cell responses against HCMV could be stimulated by either envelope glycoproteins (Liu *et al.*, 1988), tegument (Forman *et al.*, 1985) or IE proteins (Alp *et al.*, 1991). However, failure of the essential viral proteins to associate with MHC molecules resulted in a low T cell proliferation responses (Schwartz, 1985; Liu *et al.*, 1993). Thus, impaired or depleted $CD4^+$ T cells will certainly produce negative effects on the immune response against virus infection (Allan *et al.*, 1990). It was also reported that viruses such as HCMV can produce proteins, which bind to β-microglobulin of MHC class I protein and effectively inhibit the expression of class I protein on the cell membrane (Koszinowski *et al.*, 1990). Therefore, MHC class I-restricted $CD8^+$ Tc cell responses will effectively be impaired. However, whether this type of mechanism operates in EHV-1 infection is unknown.

Normally, $CD8^+$ Tc cells are able to kill virus-infected cells via MHC class I recognition resulting in

elimination of the infectious virus. Many viruses have been shown to elicit this type of response especially during the process of recovery (Larsen *et al.*, 1983; Ada and Jones, 1986). It was reported that the clearance of infectious viruses in mouse lung was mediated by $CD8^+$ T cells such as in the RSV infection (Taylor *et al.*, 1985). A similar mechanism appeared to be related to the protection observed in EHV-1 infection. In a separate experiment using spleenic cells obtained from EHV-1 infected mice, a specific killing of 3T3 and LM cell targets infected with EHV-1 has been demonstrated using CTL assay (Tewari, pers. comm.). However, high level of these cells during early infection may cause severe lung damage leading to respiratory distress as observed in the RSV infection in mice (Cannon *et al.*, 1988).

EHV-4 was found to be non-pathogenic in mice. It, nevertheless, elicited antibody and strong DTH responses to EHV-1 antigens. Thus, there is a cross-reaction between EHV-4 and EHV-1. Intranasal immunisation of mice with live EHV-4 was found to confer protection against challenge with EHV-1. This protection was related to the DTH and correlated with the T-cell mediated immunity by means of adoptive transfer of immune spleen cells. Thus, the protection conferred was found to be mediated by immune T cells and not by antibody responses alone. Since EHV-4 was not found to replicate in the respiratory tissues, it is intriguing that the virus can nevertheless elicit protective cell-mediated immune responses against the heterologous EHV-1 infection, and at the same time, perhaps, mediate immunopathology in the lung. It should be notable that immunisation with inactivated EHV-4 was not protective and in this respect was similar to immunisation with inactivated EHV-1.

For many herpesviruses, immunisation with purified virus-specific glycoproteins was found to be protective. For example, individual HSV-1 glycoproteins invoked protective antibody and cell-mediated responses (Blacklaws *et al.*, 1990; Ghiasi *et al.*, 1992). Purified gB was found to be more effective in invoking cell-mediated immune responses compared to gD or gH (Chan *et al.*, 1985), but a cell line expressing gD induced stronger antibody and CMI than those with gB (Blacklaws *et al.*, 1987). In horses, serum from EHV-1-infected animals was shown to immuno-precipitate the high abundance glycoproteins (Allen and Bryans, 1986). Recently, immunisation with baculovirus expressing EHV-1 gD was found to be protective in mice; both humoral and cellular responses were demonstrated. The EHV-1 L-particles, which were thought to contain all known envelope glycoproteins including those mentioned above, were used to immunise mice and foals intranasally. Protection, associated with cellular immune responses by means of DTH, to EHV-1 infection was demonstrated (Azmi, 1994). Based on the findings reported on the immunisation against EHV-1 using individual recombinant glycoproteins, the use of purified L-particles is likely to be protective (Azmi, 1994). Nevertheless, to date, none of these vaccine strategies appear to offer an effective measure to combat latent and reactivating EHV-1 and EHV-1 remains a problem similar to that posed by other herpesviruses (Donnenberg *et al.*, 1980; Nash, 1981; Nash and Cambouropoulos, 1993).

Other immune responses against EHV-1

Complement mediated antibody response

Other studies on EHV-1, however, have concluded that high levels of neutralising antibody can offer protection. For example, protection was demonstrated in mice following passive immunisation with neutralising hyperimmune sera (Awan *et al.*, 1990) or in hamsters by the neutralising monoclonal antibodies (Stokes *et al.*, 1989; Stokes *et al.*, 1991a). Protection observed in these studies, however, may not be mediated by the neutralising antibody *per se*, but could have involved indirect effects such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement mediated lysis by the classical pathway, which have been shown for other virus infections (Perin *et al.*, 1976). Additionally, the ability of neutralising antibody was reported to be enhanced by 8-32 folds in the presence of complement (Horimoto *et al.*, 1989; Azmi, 1994).

Antibody-dependent cell-mediated cytotoxicity

Neutrophils and mononuclear cells were shown to mediate ADDC in horses (Fujimiya *et al.*, 1979; Stokes and Wardley, 1988). Other cells such as NK, K and certain T cells may act as effectors in ADDC, as demonstrated for HSV-1 (Shore *et al.*, 1976). This mechanism has been shown to mediate lysis of EHV-1-infected cells (Wilks and Coggins, 1977a). Following EHV-1 infection in horses, the response was first detected at day 14 p.i. and peaked at day 29 p.i. (Chong *et al.*, 1992). Thus, the ADDC mechanism was suggested to be potentially important during the process of recovery and may, of course, be regulated by γIFN.

Monocytes

In addition to $CD4^+$ and/or $CD8^+$ T cells, other accessory cells including monocytes and macrophages have also been shown essential for enhancement of cell-mediated responses (Stohlman, 1991). Immediately after BHV-1 infection in cattle, the number of cells in the lungs and draining lymph nodes expressing MHC

class II was increased. This increase was correlated with an increase in the number of monocyte/macrophages in PBL and lungs (Moskophidis *et al.*, 1989). The MHC class II-restricted $CD4^+$ T cell responses in the lung could then be enhanced. Monocyte function was shown to increase during the EHV-4 infection but impaired during acute EHV-1 infection. This was suggested to be due to the EHV-1 infection of the peripheral blood mononuclear cells (Bridges and Edington, 1986).

Interferon (IFN)

IFN is secreted by many cell types following viral infection, particularly by the virus-infected cells. The release has indirect effects on the replication of many viruses. In some cases, it acts by protecting uninfected cells from penetration by virus. The γIFN is among the lymphokines secreted by T cells (and also by NK cells), which acts on macrophages and effector cells in ADDC.

Monocytes and lymphocytes of EHV-1 or EHV-4-infected horses produce IFN without any impairment. However, EHV-1 infection induces quantitatively more IFN than EHV-4 infection. The IFN was found to be released into the nasal secretions following infection by both EHV-1 nd EHV-4 but serum IFN was detected only in EHV-1 infection (Bridges and Edington, 1986; Edington *et al.*, 1989). Since IFN can reduce virus spread by ∝ and β IFNs and modulates the immune responses (γIFN), the antiviral effects mediated by IFN are probably of value in the process of recovery from EHV-1 or EHV-4 infections.

IMMUNOSUPPRESSION

A possible explanation for the low antibody responses following primary i.n. inoculation of mice with live EHV-1 was the immunosuppression. Evidences have shown that immunosuppression was not restricted to the antibody response alone but also in lymphocyte (Mohd-Azmi and Field, 1997) and antigen presenting cells (APCs) (Siedek *et al.*, 1999).

The cell-type that appeared to carry the EHV-1 infectious virus in the blood of mice was the T cells. Since the antibody response to many herpesviruses is generally thought to be of T cell-dependant, interference with the normal T cell functions would have consequential effects on the B cell functions (Mosmann and Coffman, 1989; Koszinowski *et al.*, 1991) leading to the possible decreased in antibody production.

Despite a relatively high neutralising antibody levels produced in EHV-1-infected horses (Gibson *et al.*, 1992b), suppressive effects have been observed. This was evidenced by a marked depression in the numbers of T and B cells (Hannant *et al.*, 1991). However, the suppressive effects were not obvious when horses were infected with an attenuated strain or a TK^- mutant (Slater *et al.*, 1993). Furthermore, the $CD4^+$ and $CD8^+$ T cells from EHV-1-infected horses were reported to be EHV-1-positive during the viremic phase of the infection (Slater *et al.*, 1994). Thus, a direct infection of T cells by EHV-1 may be responsible for the suppression of T cells.

Immunosuppression has also been reported in several other viral infections. Cattle infected with BHV-1 were reported to have impaired T-cell responses (Miller-Edge and Splitter, 1986) as were mice infected with influenza virus (Leung *et al.*, 1980). In many cases, a lymphocyte proliferation response by Con A or IL-2 was also suppressed (Carter *et al.*, 1989). In this context, it was important to define potential glycoproteins and their epitopes, which might immunosuppress or fail to stimulate T cell function and/or antibody production. This information is important in order to design an improved vaccine. Curtsinger *et al.* (1994), reported that following infection by HCMV, certain individuals lack antibody response to a certain portion of gB (gp93), suggesting a lack of specific T_H responsiveness required to help antibody production. It was suggested that the failed response may be due to the immunogenetic differences in the MHC genes (Liu *et al.*, 1993; Curtsinger *et al.*, 1994) that encodes class II molecules, which are required to present antigenic peptides on the cell membrane for recognition by T cells.

IMMUNISATION

A major problem concerning vaccination against EHV-1 and EHV-4 in horses is the ability of these viruses to remain latent, invades the immune surveillance and then reactivates (Edington *et al.*, 1985; Browning *et al.*, 1988). Therefore, the ideal vaccine should be designed to give life-long immunity, protecting animals from clinical problems caused by both EHV-1 or EHV-4 and be capable of preventing latency and or reactivation. Vaccination of horses with a live TK^- EHV-1 mutant either intramuscularly or intravenously was one approach that has been considered. However, despite a significant rise in serum neutralising antibody, the vaccinated horses were not fully protected and failed to prevent hyperthermia, viraemia and shedding of virus (Cornick *et al.*, 1990; Slater *et al.*, 1993).

Several different commercial vaccines are available either in live, modified live or inactivated form (Crandell *et al.*, 1980). These vaccines induce

neutralising antibody to both EHV-1 and EHV-4 and probably decreases the virus shedding, but failed to fully protect horses from developing respiratory signs and abortions (Burrows *et al.*, 1984; Mumford and Bates, 1984). Several immunising doses, which are needed for the protection due to the short-lived immunity can be overwhelmed by a high dose of infection (Thompson *et al.*, 1979). In conclusion, the available vaccines are generally considered to be unsatisfactory (Burki *et al.*, 1990).

Although both EHV-1 and EHV-4 were reported to be antigenically cross-reactive (Flowers and O'Callaghn, 1992; Azmi and Field, 1993b; Azmi and Field, 1995), only one way protection was evidenced. Immunisation with EHV-4 did not protect horses from EHV-1, but immunisation with EHV-1 protected horses from EHV-4 (Edington and Bridges, 1990). Since the experimental horses were of unknown infection status prior to the experiment, the interpretation of these observations suffers from the problem of possible pre-exposure to one or both viruses. Nevertheless, the findings may be questioned by the evidence of type-specific neutralising antibody in experimentally infected SPF foals (Gibson *et al.*, 1992b).

EPILOGUE

Studies in mouse models have provided a new insight regarding the nature of host defense mechanisms against EHV-1 infection. This provides clues to the disease in the natural host. It helps in identifying the likely mechanisms of protection to EHV-1 infection in relation to the natural disease in horses. It is evidenced that the immune cells rather than antibody responses mediated the important protection against EHV-1 infection. The mouse model was found to be useful in studying EHV-1 infection with great potential for deeper studies involving molecular aspects of immunology such as mapping of T cell or B cell epitopes derived from particular immunogenic glycoproteins (Alwan and Openshaw, 1993).

The $CD8^+$ and $CD4^+$ cells were shown to be important for protection as demonstrated by the T cell-depletion studies. Local immune responses were also important and therefore, the role of $CD4^+$, $CD8^+$, T cells and other T cells in mediating immune responses in the respiratory tract is intriguing. Other cells such as NK cells, K cells and monocytes, together with local antibodies and IFN, were important for the recovery from EHV-1 infection. As shown in HSV-1, different epitopes on the same or different glycoproteins can elicit different types of immune responses (Connors *et al.*, 1992). Some can elicit antibody responses while others elicit T cell ($CD4^+$ and/or $CD8^+$) responses. It is challenging to define those specific epitopes. When the immunodominant epitopes are defined, T cell lines/clones can be established and analysed functionally (Luckacher *et al.*, 1984; Taylor *et al.*, 1990). Then, immunising animals with appropriate peptides can induce the required immune responses.

Following virus inoculation, the lungs are normally infiltrated by many cells, which are responsible for protection of the lungs. However, at the same time they may lead to immunopathology.

The mechanism of immunosuppression following EHV-1 infection involves the virus infection of lymphocytes and probably the monocytes. However, the mechanism underlying the suppression of lymphocyte function is unknown.

It has been shown that the genetic background has significant effects on the processing and presentation of viral antigens by the antigen presenting cells, which lead to different patterns of immune response (Curtsinger *et al.*, 1994). Recently, it has been shown that CBA (H-2K) mice infected with EHV-1 strain AB4 produce higher antibody response as compared to BALB/c (H-2D) mice. A similar observation was also reported in C3H mice infected by EHV-1 strain AB3 (R.A. Killington, pers. comm.). Therefore, different virus and mouse strains should be used to investigate this phenomenon and possibly that different epitopes on the same glycoprotein may produce different immune response patterns. This may lead to a better understanding of intra- or inter-strain host variation in terms of immunogenicity and protection in horses.

Mice immunised with EHV-4 were protected from EHV-1 infection (Mohd-Azmi, 1999b). However, the mechanism by which the non-replicative EHV-4 induced protection is unknown. It is suggested that EHV-4 undergone abortive infection by producing and presenting the virus proteins without the release of infectious virus. In theory, the viral antigens could be processed in APCs, transported and presented on the cell membrane in association with MHC class I or class II membrane proteins and trigger MHC-restricted T cell responses as discussed previously. However, this has yet to be proven.

The EHV-4 expresses many glycoproteins, which cross-react with those of EHV-1. However, EHV-4 is non-pathogenic in mice, and it is intriguing to know which glycoproteins are important in modifying the EHV-4 capability of productive infection in mice. Different EHV-4 and EHV-1 strains could be used in studies of cross-reaction, particularly at the cellular level.

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RINGKASAN

PERKEMBANGAN DALAM KEPATOGENAN DAN IMUNOLOGI HERPESVIRUS EKUIN TIP-1

Kertas ini menyorot kepatogenan dan gerakbalas imun hos terhadap herpesvirus ekuin tip-1 yang menyebabkan rinopenumonitis, keguguran dan gangguan system sraf pusat pada kuda. Penekanan khusus diberikan kepada tindakbalas hos terhadap virus dalam model murin yang merangkumi perubahan kepatogenan major, cirian klinikal serta keimunan kepada jangkitan EHV-1. Sebelum menjalar ke peparu selepas jangkitan intranasum, EHV-1 mengukuhkan viremia terkait-sel dimana berlaku pemreplikatan secara besaran dan menyebabkan penyakit klinikal berlaku di peparu.Virus ini boleh diasingkan daripada tisu respiratori, limpa, hati, ginjal, otak dan nod limfa. Virus juga boleh merentasi plasenta untuk menjangkiti fetus yang boleh menjurus kepada keguguran atau kelahiran pramatang. Titer virus dalam otak adalah amat rendah. Haiwan yang terdedah kepada virus secara intranasum mempunyai titer antibodi yang rendah dalam serum dan rembesan respiratori. Walaupun perlindungan dilihat dalam jangkitan seterusnya. ia tidak berlaku pada mencit yang terimun melalui cara lain. Bagaimanapun, beberapa kajian menunjukkan bahawa perlindungan primer daripada jangkitan EHV-1 adalah melalui gerakbalas imun terkait-sel (gerakbalas DTH dan penyusupan $CD4^+$ pada peparu).Lanjutan daripada beberapa kajian, diramalkan bahawa aktiviti sel T sitotosik ($CD8^+$) adalah penting dalam mengawal jangkitan EHV-1 dan EHV-4. Dengan itu, dirumuskan bahawa sel $CD4^+$ dan $CD8^+$ adalah penting dalam mengawal jangkitan primer dan sekunder EHV-1. Faktor imun penting lain yang terlibat merangkumi interferon (IFN), ADCC dan gerakbalas antibodi perantaraan komplemen. Aktiviti imunotindsan EHV-1, tindakbalas silang dengan EHV-4 dan kelebihannya sebagai agen pengimunan terhadap EHV-1 diterangkan.