Immunokinetics of equine herpesvirus 1 in donkey mares: suppression of secondary cell-mediated response

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Summary: To study the immunokinetics of equine herpesvirus 1 (EHV1), donkey mares were immunised with a laboratory strain of EHV1, or with recommended doses of Pneumabort-K vaccine (EHV1 Army 183 strain, formalin inactivated, with an oil adjuvant) and a booster was given after three months.

Humoral immune responses were studied by employing a virus neutralisation (VN) test. A leucocyte migration inhibition test (LMIT) was employed for the assay of cellular immune responses.

The VN antibody titre reached 1:64 or 1:128 after primary immunisation and showed a marginal increase (1:256) after secondary immunisation with either of the immunogens.

After the primary dose of immunogen, there was a gradual increase in host cellular response which persisted for up to three months. However, on secondary immunisation, cell-mediated immune response was short-lived and weak compared to the primary response with both immunogens. This could be one possible explanation for breakdown of anti-EHV1 immunity leading to abortion in immunised mares.

KEYWORDS: Equine herpesvirus - Immune response - Immunosuppression Leucocyte migration inhibition test - Primary and secondary immune responses Virus neutralisation test.

INTRODUCTION

Equine herpesvirus 1 (EHV1) is the causative agent of respiratory infection, abortions, perinatal foal mortality and paralysis in horses. It is responsible for considerable economic loss to the horse industry world-wide (1, 7). For the prevention of EHV1 infection in horses, Pneumabort-K vaccine containing inactivated EHV1 antigen has been reported to be effective, suggesting suitability for field applications (1, 3). However, the ability of this vaccine to protect against simultaneous challenge with both EHV1 and EHV4 has been questioned by others, as it may provide only partial protection (6, 19).

The immunity of horses to EHV1 infection is characteristically short-lived and the animals become susceptible to reinfection within three to four months of recovery, especially after respiratory infection. Despite the presence of neutralising antibodies

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to EHV1 in the sera of animals following vaccination, the animals showed clinical manifestations (5, 8). It is obvious from the literature that immune mechanisms other than neutralisation by antibodies are important in protection from EHV1. The aim of the present study was to examine the humoral and cellular immune responses in donkey mares (the most closely related species to horses) upon primary and secondary immunisation with live virus and inactivated vaccine, to understand the nature of short lived immunity to EHV1.

**MATERIALS AND METHODS**

**Virus**

A local isolate of EHV1 adapted in ovine kidney cell culture containing approximately $10^6$ TCID$_{50}$/ml was used for immunisation. Before use as an antigen in the leucocyte migration inhibition test (LMIT), the virus was inactivated by exposure to ultraviolet (UV) radiation.

**Vaccine**

Pneumabort-K vaccine was used for immunisation of the animals following the recommendations of the manufacturer, i.e. 2 ml intramuscularly (i.m.).

**Experimental animals**

Apparently healthy non-pregnant donkey mares aged one to two years were purchased from a local contractor and maintained on gram fodder under conventional conditions.

**Immunisation protocol**

Three donkey mares in one group were immunised with Pneumabort-K vaccine and three in a second group with ovine kidney-adapted live virus (2 ml, i.m.). The two groups were kept separate from each other. Two donkey mares were maintained as unvaccinated controls and housed separately. After an interval of three months, the two groups of donkey mares were given a second immunisation with the same vaccine using the identical route and dose.

The donkey mares were bled at intervals after primary and secondary immunisation to obtain serum and leucocytes for virus neutralisation (VN) tests and LMIT, respectively.

**Virus neutralisation test**

The VN test was performed according to the protocol described by Mitchell and colleagues (17). Briefly, heat-inactivated serum was diluted two-fold and incubated with 100 TCID$_{50}$ for 1 h at 37°C. The serum and virus mixture was inoculated into ovine kidney culture tubes (at least three tubes per dilution of serum) and observed for 7 days. From these results, VN antibody titres of the sera were determined.

**Leucocyte migration inhibition test**

LMIT was performed using the method previously described (10, 23). The non toxic (optimal) dilution was determined for the UV-inactivated virus harvested from ovine kidney cell culture and used as antigen; this was found to be 1:4. Leucocytes
were separated from the blood of both immunised and normal donkeys by removing the erythrocytes with hypotonic shock, using chilled triple glass-distilled water (22). After washing the leucocytes twice with Hank's balanced salt solution, the leucocytes were finally reconstituted in medium 199 containing 10% precolostral calf serum and antibiotics. The viability of these leucocytes was checked by the trypan blue dye exclusion technique and found to be >95%. Migration of the leucocytes charged in capillary tubes was tested both with and without the antigen. Four capillary tubes were used on average per sample and the appropriate controls were included in the test. The percent leucocyte migration inhibition (LMI) was calculated using the following formula:

\[
\text{percent LMI} = \left( \frac{\text{area of migration of immune cells with antigen}}{\text{area of migration of immune cells without antigen}} \right) \times 100 - \left( \frac{\text{area of migration of control cells with antigen}}{\text{area of migration of control cells without antigen}} \right) \times 100
\]

**RESULTS**

Primary and secondary virus neutralisation antibody response

VN tests were carried out on the sera taken at 0, 7, 14, 21, 28 and 90 days after primary immunisation and 4, 8 and 12 days after the secondary immunisation. After primary immunisation with the Pneumabort-K or live virus, VN antibody titre averaged 64, rising to 256 after secondary immunisation. The ability of the two immunogens to elicit VN antibody responses after primary and secondary immunisation was observed to be almost identical (Table I).

**Table I**

*Virus neutralising antibody titres* of donkey mares after primary and secondary immunisation**

<table>
<thead>
<tr>
<th>Days post-immunisation</th>
<th>Pneumabort-K vaccine</th>
<th>Ovine kidney-adapted strain of EHV1</th>
<th>Non-immunised control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1:8</td>
<td>1:8</td>
<td>1:8</td>
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<tr>
<td>7</td>
<td>1:64</td>
<td>1:64</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td>1:64</td>
<td>1:64</td>
<td>ND</td>
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<tr>
<td>21</td>
<td>1:64</td>
<td>1:64</td>
<td>1:8</td>
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<tr>
<td>28</td>
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<td>1:64</td>
<td>ND</td>
</tr>
<tr>
<td>90</td>
<td>1:64</td>
<td>1:128</td>
<td>ND</td>
</tr>
<tr>
<td>Secondary</td>
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<tr>
<td>4</td>
<td>1:256</td>
<td>1:256</td>
<td>1:8</td>
</tr>
<tr>
<td>8</td>
<td>1:256</td>
<td>1:256</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>1:256</td>
<td>1:512</td>
<td>ND</td>
</tr>
</tbody>
</table>

* reciprocal of highest dilution of serum which neutralised 50% CID<sub>50</sub>
** the test was carried out with pooled sera obtained from three animals
ND, no data
Primary and secondary leucocyte migration inhibition response

Migration inhibition of peripheral leucocytes was studied at 4, 8, 12 and 16 days and 4, 8, 10 and 12 weeks after primary immunisation, and 4, 8, 12 and 20 days after secondary immunisation (Fig. 1). LMI response at four days after primary immunisation was 44.10% and 41.66% respectively in the two groups, increasing gradually to 90% at 16 days and then persisting. After secondary immunisation, the LMI values were 77.30% on day 4, rapidly declining to 20% by day 12. Results were similar in the two groups, showing a rapid and enhanced response immediately after secondary immunisation. However, the duration of response was significantly short compared to the response observed after primary immunisation, indicating a suppression of LMI response after secondary immunisation.

![Primary and secondary leucocyte migration inhibition responses](image)

**DISCUSSION**

Considering the characteristically short-lived immunity to EHV1 vaccine, an immunisation protocol which stimulates an immune response comparable to that resulting from natural infection would be highly desirable.
In the present study, the kinetics of the response to inactivated and live virus vaccines was found to be similar, with VN titres of 1:128 after primary immunisation and 1:256 after secondary immunisation. Intranasal inoculation of pregnant mares with EHV1 and vaccination with a killed (Pneumabort K) vaccine which produces a threshold level of VN antibody (> 1:80) is claimed to correlate resistance to reinfection (2, 4, 18). However, the occurrence of foetal infection and abortion despite high levels of VN antibodies suggests that protective mechanisms other than antibody were involved (12, 13).

Cell-mediated immunity (CMI) responses could be detected by LMIT as early as four days after primary immunisation, reaching a peak of 90% by 16 days. After secondary immunisation, LMI response was > 70% but decreased rapidly to < 20% by 12 days. The CMI responses to the two immunogens were also almost identical (Fig. 1). It has been observed in certain vaccine trials that some ponies were protected against infection in the absence of strong concentrations of VN antibodies (19) and CMI has been reported to play an important role in protection against EHV1 (10, 23).

If the CMI response is important for protection, then a low and/or unpredictable CMI response after repeated vaccination could be one possible reason for the failure or breakdown of EHV1 vaccination.

The suppressed (weak and short-lived) LMI responses after secondary immunisation noted in this study could have several explanations. Firstly, T cell responses have been shown to be inhibited by a normal humoral response due to the formation of antigen-antibody complexes (11). Secondly, there could be impairment in the induction of certain cytokines, such as Interleukin-2, which regulate CMI (16).

Alternatively, the induction of suppressor cells during primary immunisation may be responsible for the suppression observed after secondary immunisation (9, 14).

Many other viruses, including other herpesviruses of man and animals, are also known to be immunosuppressive, working at different levels by various mechanisms (15, 20, 21). However, the present study is particularly interesting in that although the primary response was normal, the suppression was observed only after secondary immunisation of the animals. This calls for a careful re-evaluation of the recommendations of the manufacturers to use vaccine at frequent intervals during the entire period of pregnancy against a background of frequent natural exposure due to endemic EHV1. This is all the more true if the major role in achieving the objective of protection is played by CMI. Further research is in progress, using inbred laboratory animal models (hamsters and mice) to elucidate the exact mechanism of EHV1 mediated immunosuppression and its role in predisposing animals to reinfection with EHV1 and other infectious agents.

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CINÉTIQUE DE LA RÉPONSE IMMUNITAIRE DES ÂNESSES A L’HERPÈSVIRUS 1 DES ÉQUIDÉS : SUPPRESSION DE LA RÉPONSE À MÉDIATION CELLULAIRE SECONDAIRE. — M. Singh et S. Charan.

Résumé : Dans le but d’étudier la cinétique de la réponse immunitaire à l’herpèsvirus 1 des équidés (HVIE), des ânesses ont été immunisées par une souche de laboratoire de ce virus ou par le vaccin Pneumabort-K aux doses recommandées (vaccin à adjuvant huileux, préparé à partir de la souche 183 inactivée par le formaldéhyde), avec un rappel trois mois plus tard.

La réponse immunitaire humorale a été mesurée par une épreuve de neutralisation virale et la réponse cellulaire par un test d’inhibition de la migration des leucocytes.

Un titre d’anticorps de 1:64 ou 1:128 a été atteint après la première immunisation et une augmentation marginale (1:256) après la seconde immunisation par l’un ou l’autre des immunogènes.

Après administration de la première dose d’immunogène, on a pu observer une augmentation progressive de la réponse immunitaire à médiation cellulaire de l’hôte, qui a persisté jusqu’à trois mois. Après la seconde immunisation, cette réponse était par contre de courte durée et peu marquée, comparativement à la réponse primaire aux deux immunogènes. Ce phénomène pourrait expliquer la rupture de l’immunité anti-HVIE qui provoque des avortements chez les juments immunisées.

MOTS CLÉS : Epreuve de neutralisation virale - Epreuve d’inhibition de la migration des leucocytes Herpèsvirus des équidés Immunosuppression Réponse immunitaire - Réponses immunitaires primaire et secondaire.

INMUNOCINÉTICA DEL HERPESVIRUS EQUINO 1 EN BURRAS: SUPRESIÓN DE LA RESPUESTA SECUNDARIA MEDIADA POR CÉLULAS. — M. Singh y S. Charan.

Resumen: Para estudiar la inmunocinética del herpesvirus equino 1 (EHV1), se inmunizaron unas burras con una cepa de laboratorio de EHV1 o con las dosis recomendadas de vacuna Pneumabort K (cepa EHV1 Ejército 183, atenuada por formalina, con adyuvante oleoso) y se revacunaron tres meses después.

Se estudiaron las inmunorrespuestas humorales empleando una prueba de neutralización del virus (NV) y se utilizó una prueba de inhibición de migración de los leucocitos para la determinación de las inmunorrespuestas celulares.

Después de la inmunización primaria, el título del anticuerpo NV alcanzó 1:64 ó 1:128 y mostró un aumento marginal (1:256) después de la inmunización secundaria con cualquiera de los immunógenos.

Se comprobó un aumento gradual en la inmunorrespuesta celular después de la primera dosis de immunógeno que persistió hasta tres meses. No obstante, en la inmunización secundaria, la inmunorrespuesta celular fue de corta duración y débil en comparación con la respuesta primaria con ambos immunógenos. Esta podría ser una explicación posible del fracaso de la inmunidad anti-EHV1 conducente al aborto en hembras inmunizadas.
PALABRAS CLAVE: Herpesvirus equino - Inmunosupresión Prueba de inhibición de la migración leucocitaria - Prueba de neutralización del virus Respuesta inmune Respuestas inmunes primaria y secundaria.

REFERENCES


