Effect of Age and Pregnancy on the Antibody and Cell-mediated Immune Responses of Horses to Equine Herpesvirus 1

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ABSTRACT

The cell-mediated immune response and antibody response of horses of varying ages and of pregnant horses to equine herpesvirus 1 antigen were examined. Six to eight month old horses showed either no increase or slight increases in anti-equine herpesvirus 1 serum neutralizing antibody following vaccination and revaccination with a modified live equine herpesvirus 1 vaccine. However, these same horses showed a marked increase in the cell-mediated immune response to equine herpesvirus 1 as measured by the lymphocyte transformation test. Eighteen to 21 month old horses showed four to 64-fold increases in anti-equine herpesvirus 1 serum neutralizing antibody titer following vaccination, but the cell-mediated immune response to equine herpesvirus 1 was low or absent. Only after revaccination did they show an increased cell-mediated immune response to equine herpesvirus 1. The cell-mediated immune response of mares in the latter stages of pregnancy to equine herpesvirus 1 was suppressed although antibody titers increased as much as 16-fold following exposure to virulent equine herpesvirus 1.

RÉSUMÉ

Cette expérience visait à étudier l'immunité cellulaire et humorale, à l'endroit du type 1 du virus herpes équin, chez des cheveaux de différents âges et des juments en gestation. Deux injections d'un vaccin atténué à des sujets âgés de six à huit mois ne produisirent aucune immunité humorale ou seulement une faible augmentation d'anticorps sériques neutralisants. Ces mêmes chevaux développèrent cependant une bonne immunité cellulaire, comme le révéla l'épreuve de la transformation des lymphocytes. La vaccination des sujets âgés de 18 à 21 mois se traduisit par le développement d'anticorps sériques dont le titre variait de 1:4 à 1:64; leur immunité cellulaire se révéla cependant basse ou nulle, mais elle accusa une hausse après une revaccination. L'immunité cellulaire des juments en gestation avancée se trouva supprimée; leur contact avec une souche virulente du type 1 du virus herpes équin se traduisit cependant par le développement d'anticorps sériques dont le titre atteignit 1:16.

INTRODUCTION

Viral rhinopneumonitis is a disease caused by equine herpesvirus 1 (EHV 1) (3). This highly contagious virus infects the mucous membranes of the upper respiratory tract. Secondary bacterial infection often follows with mucopurulent rhinitis, pharyngitis and persistent coughing (4). Equine rhinopneumonitis seldom leads to death but can cause neurological disorders (14) and abortions (6).

Infection of horses with EHV 1 is generally followed by the appearance of anti-EHV 1 antibody. However, the presence of anti-EHV 1 antibody in pregnant mares is not always associated with protection against reinfection (3) nor is the lack of development of high titers to anti-EHV 1 antibody necessarily associated with susceptibility to disease (6, 7).

Wilks and Coggins (25, 26) and Pachciarz (20) demonstrated that horses also develop a cell-mediated immune (CMI) res-

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ponse to EHV 1 following experimental infection with EHV 1. Since the nature of the herpesvirus-host relationship is such that the virus spreads from infected cells to susceptible cells without exposure to specific antibody, the CMI response may be the most important defense against herpesvirus dissemination (19). In addition, abortions in broodmares can result from dissemination of EHV 1 in the presence of high serum neutralizing (SN) antibody titers to EHV 1 (3). Insofar as resistance to infection of EHV 1 is not adequately explained in terms of antibody titers, development of a CMI response to EHV 1 may be of paramount importance in protecting horses against infectious EHV 1.

In the present study, we show that horses develop a CMI response to EHV 1 following vaccination with a modified live (ML) EHV 1 vaccine.[1] However, the genesis of this response, in conjunction with the antibody response to EHV 1, differs between young horses six to eight months of age and horses 18 to 21 months of age. We also show how pregnancy affects the CMI response of previously vaccinated mares following exposure to virulent EHV 1. The relevance these findings might have in the protection of the horse against infectious EHV 1 is discussed.

MATERIALS AND METHODS

Vaccinal Antigens

The vaccinal strain of EHV 1 was grown in tissue culture on an established equine 4 cell line.[2]

Preparation of Test Antigens

The live EHV 1 lymphocyte transformation test antigen was prepared in the same manner as the vaccinal antigen without the addition of the vaccine stabilizer. The EHV 1 antigen contained virus and cell membrane material. Infectious bovine rhinotracheitis (IBR) virus was grown on an established bovine kidney cell line in the same manner as the EHV 1 antigen. The EHV 1 antigen was inactivated by heating at 56°C for one hour. Control EHV 1 and IBR viral antigens were noninfected equine 4 and bovine kidney 3[2] cell line fluids respectively. Culture medium consisted of RPMI 1640[3] containing 1% penicillin-streptomycin[4] and 1% L-glutamine.[5]

Animals

Six male and 13 female horses born at Norden Laboratories and varying in age from six to 21 months were vaccinated. Two additional male and three additional female horses were nonvaccinated controls. None of the horses were previously given EHV 1 vaccine.

Immunization Procedure

Eleven of the vaccinated horses were 18 to 21 months of age and eight horses were six to eight months of age at the time of intramuscular vaccination with a ML-EHV 1 vaccine. The horses were revaccinated after four weeks. Blood samples were collected from the test animals before the first vaccination, at one and four weeks following vaccination and at four week intervals following revaccination. Two to four months following vaccination, six of the mares became pregnant and subsequently gave birth to normal foals.

Field Infection

Field infection with virulent EHV 1 in all five nonvaccinated controls, in five of six EHV 1 vaccinated stallions and ten of 13 vaccinated mares available for experimentation was ascertained by four to 16-fold increases in SN titers to EHV 1 of nonvaccinated control horses (Table III). Exposure of horses to virulent virus occurred two to five months prior to the birth of foals.

[1]Rhinomune, Norden Laboratories, Lincoln, Nebraska.

[2]Norden Laboratories, Inc., Lincoln, Nebraska.

[3]Flow Laboratories, Rockville, Maryland.

[4]Grand Island Biological Company, New York, New York.

[5]General Chemical, Chagrin Falls, Ohio.

Lymphocyte Transformation Test

Twenty ml of blood was collected by venipuncture in heparinized tubes (25 IU of heparin/ml) and allowed to sediment at room temperature for 20 minutes. Triplicate 0.1 ml samples containing 6 x 10^5 to 1.2 x 10^6 leukocytes in autologous serum were mixed with 1.4 ml amounts of viral antigen, control fluids, or RPMI control medium. It was determined that 4.5 to 6 x 10^4 plaque forming units of EHV 1 per ml induced the greatest lymphocyte response. The samples, in loosely capped glass tubes, were incubated for six days at 37°C in a humidified 5% CO_2 atmosphere. On the sixth day, one μCi ^{3}H-thymidine[6] (specific activity, 20 Ci/mMole) in 0.5 ml of culture medium was added to all cultures receiving antigen, control fluids or medium alone and incubated an additional 16 hours. Following incubation, three ml aliquots of 3% glacial acetic acid were added to each tube. The mixture was filtered by negative pressure through GF/A glass filter paper[7] cut to a diameter of 13 mm. The filter was washed with 3% glacial acetic acid and transferred to a glass scintillation vial containing five ml of Aquasol solubilizer[8]. The radioactivity of each sample was measured by liquid scintillation spectrometry. Data were reported as net disintegrations per minute (DPM) or as the stimulation index (SI). Net DPM were determined by subtracting the DPM of control fluids from the DPM of stimulated cultures. A SI $\geq$3.0 was considered a positive CMI response to EHV 1. The stimulation index was determined as follows:

$$\frac{\text{DPM, stimulated cultures - blanks}}{\text{DPM, unstimulated cultures - blanks}} = \text{SI}$$

Antibody Titrations

The anti-EHV 1 SN antibodies were measured by a microtitration test using equine fetal kidney cells and the 80% end point determined. Tests were repeated periodically with reproducible results.

[6]New England Nuclear, Boston, Massachusetts.

[7]Whatman, W. R., Balston, Ltd., England.

[8]New England Nuclear, Boston, Massachusetts.

Statistical Analysis

Results were analyzed by the t-statistic test.

RESULTS

CMI Response to EHV 1

Lymphocytes from EHV 1 vaccinated and nonvaccinated horses were cultured with live and inactivated EHV 1 cell-associated antigens and with live IBR cell-associated antigen in order to test the sensitivity and specificity of lymphocyte stimulation by EHV 1. The lymphocyte response is directed to EHV 1 antigenic determinants and not antigenic determinants of cell membrane fragments as demonstrated by the relatively low responsiveness or lack of responsiveness to control fluids (Table I). Live EHV 1 suppressed the response of nonimmune lymphocytes from nonvaccinated horses to EHV-1 (SI $\leq$1.0) but not the response of immune lymphocytes from vaccinated horses (Figs. 1). Lymphocytes from nonvaccinated horses showed some response to inactivated EHV 1. However, these responses, which may be a mitogen effect of the inactivated EHV 1,

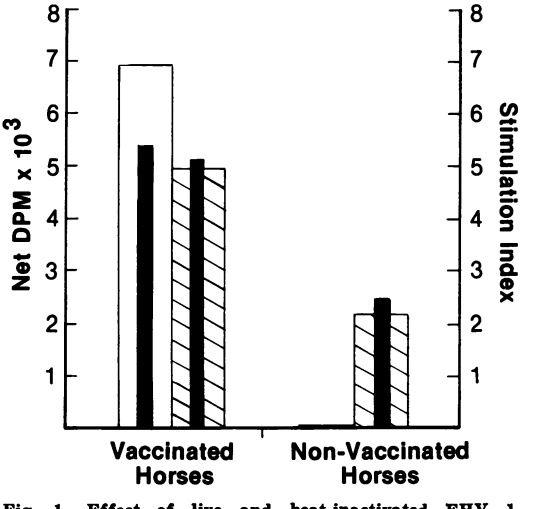

Fig. 1. Effect of live and heat-inactivated EHV 1 on the in vitro lymphocyte response of vaccinated and nonvaccinated horses to EHV 1. ☐ = live EHV 1, ▨ = heat-inactivated EHV 1, ■ = Stimulation Index, Ratio of DPM, EHV 1 cultures/DPM, control fluids.

were not considered significant (SI ≤3.0). In contrast, the lymphocyte response of vaccinated horses was similar both to live and inactivated EHV 1. Since live EHV 1 did not increase or suppress lymphocyte transformation in vaccinated horses and discriminated better than inactivated EHV 1 between lymphocyte responsiveness of vaccinated and nonvaccinated horses, live EHV 1 virus was used in all tests reported below. Lymphocytes from horses were not stimulated by live IBR virus suggesting

TABLE I. EHV 1 Stimulation of Lymphocytes from EHV 1 Vaccinated and Nonvaccinated Horses

	Horse No.	^{3}H-Thymidine Incorporation (dpm ± SE x 10^3) Media	Control Fluid	EHV 1	Specific Increase In ^{3}H-Thymidine Incorporation (dpm ± SE x 10^3) EHV 1 — Control Fluid
	1	0.5 ± 0.0	0.8 ± 0.1(1.6)[a]	8.9 ± 1.3(11.2)[b]	8.1 ± 1.2
	2	1.0 ± 0.3	1.2 ± 0.3(1.2)	14.1 ± 4.3(11.8)	12.9 ± 4.0
	3	0.8 ± 0.1	1.5 ± 0.0(1.9)	6.1 ± 0.5(4.1)	4.6 ± 0.5
V	4	0.9 ± 0.0	1.1 ± 0.0(1.2)	5.1 ± 0.1(4.6)	4.0 ± 0.1
A	5	0.5 ± 0.0	1.6 ± 0.2(3.2)	6.1 ± 0.7(3.8)	4.5 ± 0.5
C	6	0.3 ± 0.0	1.7 ± 0.1(5.6)	3.8 ± 0.2(2.2)	2.1 ± 0.1
C	7	1.0 ± 0.2	2.5 ± 0.5(2.5)	12.1 ± 1.1(4.9)	9.7 ± 0.6
I	8	0.6 ± 0.0	2.6 ± 0.0(4.2)	24.8 ± 4.5(9.5)	22.2 ± 4.5
N	9	2.6 ± 0.9	2.0 ± 0.5(0.8)	9.8 ± 0.8(4.9)	7.8 ± 0.3
A	10	1.5 ± 0.0	2.4 ± 0.6(1.6)	11.7 ± 1.8(4.9)	9.3 ± 1.2
T	11	0.6 ± 0.0	4.6 ± 0.2(7.7)	9.3 ± 0.4(2.0)	4.7 ± 0.2
E	12	0.5 ± 0.0	3.8 ± 0.0(7.6)	11.9 ± 1.0(3.1)	8.1 ± 1.0
S	13	2.5 ± 0.0	9.5 ± 0.5(3.8)	131.3 ± 13.3(13.8)	121.8 ± 12.8
	14	0.5 ± 0.1	1.4 ± 0.4(2.1)	9.8 ± 0.9 (7.0)	8.4 ± 0.5
C					
O					
N	15	0.7 ± 0.1	1.5 ± 0.1(2.1)	1.4 ± 0.1(0.9)	0.0 ± 0.0
T	16	ND	1.7 ± 0.1(ND)	1.7 ± 0.1(1.0)	0.0 ± 0.0
R	17	0.5 ± 0.0	1.7 ± 0.0(3.4)	1.5 ± 0.0(0.9)	0.0 ± 0.0
O	18	0.4 ± 0.0	1.6 ± 0.0(4.0)	1.7 ± 0.0(1.1)	1.0 ± 0.1
L	19	0.8 ± 0.0	1.9 ± 0.0(2.4)	1.6 ± 0.0(0.8)	0.0 ± 0.0
S					

[a]Stimulation index. Ratio of control fluids/media
[b]Stimulation index. Ratio of EHV-1/control fluids

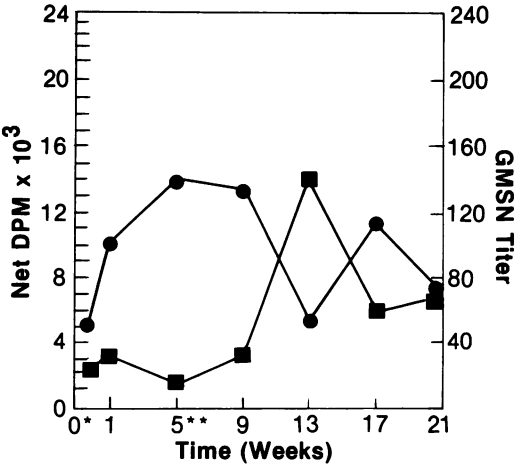

Fig. 2. Cell-mediated immune response and antibody response to EHV 1 of 11 horses, 18-21 months of age, given ML-EHV 1 vaccine. ■ = mean net disintegrations per minute (DPM), ● = reciprocal of the geometric mean serum neutralization titer (GMSN), * = pre-1st vaccination, ** = pre-2nd vaccination.

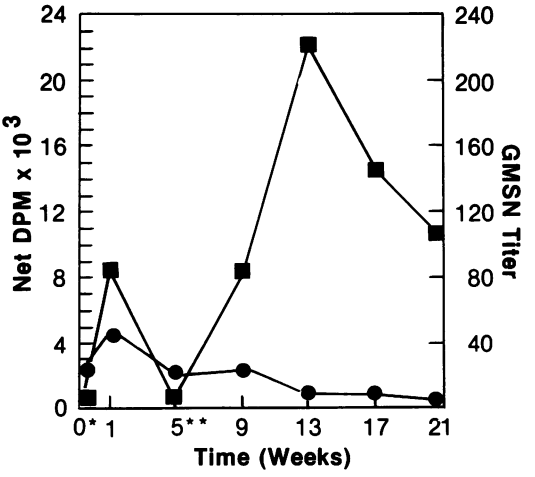

Fig. 3. Cell-mediated immune response and antibody response to EHV 1 of eight horses, six to eight months of age, given ML-EHV 1 vaccine. ■ = mean net DPM, ● = reciprocal of the GMSN titer, * = pre-1st vaccination, ** = pre-2nd vaccination.

TABLE II. IBR Virus Stimulation of Lymphocytes from EHV1 Vaccinated and Nonvaccinated Horses

	Horse No.	^{3}H-Thymidine Incorporation (dpm ± SE x 10^3) Media	Control Fluids	IBR Virus	Specific Increase in ^{3}H-Thymidine Incorporation (dpm ± SE x 10^3) IBR Virus Control Fluids
	1	0.5 ± 0.0	1.5 ± 0.0(3.0)[a]	1.8 ± 0.0(1.2)[b]	0.3 ± 0.0
	2	1.0 ± 0.3	1.8 ± 0.0(1.8)	1.8 ± 0.0(1.0)	0.0 ± 0.0
V	3	0.8 ± 0.1	1.7 ± 0.0(2.1)	1.7 ± 0.1(1.0)	0.0 ± 0.0
A	4	0.9 ± 0.0	4.7 ± 0.0(5.2)	1.6 ± 0.0(0.3)	0.0 ± 0.0
C	5	0.5 ± 0.0	1.9 ± 0.0(3.8)	1.6 ± 0.1(0.8)	0.0 ± 0.1
C	6	0.3 ± 0.0	1.9 ± 0.2(6.3)	1.6 ± 0.0(0.8)	0.0 ± 0.2
I	7	1.0 ± 0.2	2.1 ± 0.0(2.1)	2.5 ± 0.6(1.2)	0.4 ± 0.6
N	8	0.6 ± 0.0	2.5 ± 0.1(4.2)	1.8 ± 0.1(0.7)	0.0 ± 0.0
A	9	2.6 ± 0.9	5.1 ± 0.5(1.9)	2.4 ± 0.2(0.5)	0.0 ± 0.0
T	10	1.5 ± 0.0	2.5 ± 0.4(1.7)	1.7 ± 0.0(0.7)	0.0 ± 0.0
E	11	0.6 + 0.0	1.7 ± 0.1(2.8)	1.7 ± 0.1(1.0)	0.0 ± 0.0
S	12	0.5 ± 0.0	1.9 ± 0.1(3.8)	1.8 ± 0.1(1.0)	0.0 ± 0.0
	13	2.5 ± 0.0	4.1 ± 0.4(1.6)	1.7 ± 0.1(0.3)	0.0 ± 0.0
	14	0.5 ± 0.1	1.8 ± 0.7(3.6)	1.6 ± 0.1(0.9)	0.0 ± 0.0
C					
O					
N	15	0.7 ± 0.1	1.9 ± 0.0(2.8)	1.5 ± 0.2(0.6)	0.0 ± 0.0
T	16	ND	1.7 ± 0.0(ND)	1.8 ± 0.1(1.1)	0.1 ± 0.1
R	17	0.5 ± 0.0	1.8 ± 0.2(3.6)	1.6 ± 0.2(0.9)	0.0 ± 0.0
O	18	0.4 ± 0.0	1.4 ± 0.0(3.5)	1.4 ± 0.1(1.0)	0.0 ± 0.0
L	19	0.8 ± 0.0	1.7 ± 0.0(2.1)	1.6 ± 0.1(0.9)	0.0 ± 0.0
S					

[a]Stimulation index. Ratio of control fluids/media
[b]Stimulation index. Ratio of IBR virus/control fluids

TABLE III. Effect of Pregnancy on Lymphocyte Sensitivity to EHV 1

	Stimulation Index					Serum Neutralization Titer				
		Postinfection (weeks)					Postinfection (weeks)			
Horses	Pre-infection	1	4	8	12	Pre-infection	1	4	8	12
Nonpregnant										
1	1.9	3.4	1.9	3.3	1.0	128	256	128	64	512
2	2.3	3.9	1.6	2.1	1.8	128	512	128	256	128
3	2.0	6.1	1.3	2.6	1.0	64	128	128	32	32
4	2.2	4.9	1.0	3.4	1.4	128	128	256	64	64
5	7.0	10.4	2.9	4.2	5.1	64	256	256	64	64
6	2.6	3.4	3.9	4.8	11.0	32	256	256	256	256
7	1.8	5.5	3.1	1.0	2.1	0	1024	512	1024	256
8	3.0	8.5	1.6	3.7	3.3	32	64	128	64	64
9	4.8	4.0	2.2	2.8	4.2	64	128	256	128	128
Pregnant										
10	1.2	1.8	2.6	2.9	2.3	64	128	256	128	64
11	1.8	1.0	1.5	1.4	2.3	128	128	256	128	256
12	2.0	2.2	1.0	1.4	1.0	16	64	256	64	128
13	4.5	2.9	1.0	1.8	1.1	256	512	256	512	256
14	1.0	1.0	1.3	1.3	1.6	2	8	16	8	4
15	4.3	5.6	3.9	2.6	3.6	32	64	256	64	32
Controls										
16	1.2	3.2	1.0	1.0	1.0	8	32	64	32	16
17	1.4	1.0	1.0	1.0	1.0	4	8	64	8	8
18	4.3	4.1	1.0	1.0	1.0	8	16	32	16	8
19	1.5	1.2	1.0	1.0	1.0	8	64	64	64	32
20	1.8	6.9	1.0	1.0	1.0	4	16	32	16	16

Horses numbered 5, 6, 7, 8, 9, 19 and 20 are stallions
The response of lymphocytes from pregnant horses was significantly less than the response of lymphocytes from nonpregnant horses at one week ($p < 0.01$) and eight weeks ($p < 0.025$) postinfection

that the response to live EHV 1 virus is specific (Table II).

Age and the Immune Response to EHV 1

Development of the antibody and CMI response of horses to EHV 1 was different in each age group of horses. Horses 18 to 21 months of age at the time of vaccination demonstrated two to eightfold increases in SN titers following vaccination (Fig. 2). Increased lymphycyte sensitivity to EHV 1 did not occur until eight weeks after revaccination (13 weeks postvaccination) at which time the titer of anti-EHV 1 antibody declined precipitously. In contrast, horses six to eight months of age showed low prevaccination SN titers and failed to develop high levels of anti-EHV 1 antibody following vaccination and revaccination (Fig. 3). However, these horses showed a positive CMI response to EHV 1 following vaccination and a marked increase in CMI to EHV 1 following revaccination.

Pregnancy and the CMI Response to EHV 1

Pregnancy had no suppressive influence on the humoral immune response to EHV 1 (Table III). Antibody titers in both pregnant and nonpregnant mares increased two to eightfold following exposure to infectious EHV 1. However the CMI response to EHV 1 of pregnant horses was significantly lower than nonpregnant horses ($p<0.01$ at one week and $p<0.025$ at eight weeks postinfection). Lymphocytes from five of six pregnant horses failed to show increased sensitivity to EHV 1 following exposure to virulent EHV 1 while lymphocytes from all stallions, nonpregnant mares and two of the five nonvaccinated control horses showed an increase in the incorporation of ^{3}H-thymidine following stimulation by EHV 1.

The CMI response to EHV I of vaccinated pregnant horses was, however, greater than the CMI response of nonvaccinated, nonpregnant horses. Although the lymphocyte response to EHV 1 was not significantly greater than that of control horses at one week postinfection, it was significantly greater at four weeks ($p<0.10$), eight weeks ($p<0.01$) and at 12 weeks ($p<0.025$) postinfection.

DISCUSSION

In this study, we employed *in vitro* test conditions which approximated *in vivo* situations. These test conditions included: 1) the use of nonseparated leukocytes so that a complete population of immune lymphocytes associated with other leukocyte types would be represented, 2) the use of autologous plasma so that serum factors that influence the immune responses, e.g. lymphokines (24), thymus hormones (13,21) and antibody (1,2) would be present and 3) the use of live cell-associated EHV 1 as our test antigen which neither suppressed nor stimulated lymphocyte blastogenesis and thymidine uptake apart from antigenic stimulation.

It was important to examine the ability of the young horse to develop a CMI response to EHV 1 since young horses often show no significant antibody response or only a weak antibody response to EHV 1 following vaccination (4,7). However, these horses often remain free of EHV 1 infections for up to six months despite the absence of anti-EHV 1 antibody (7).

In this study, horses that were six to eight months of age at the time of vaccination developed little or no antibody response to EHV 1 but did develop a strong CMI response to EHV 1 (Fig. 3). In contrast, horses that were 18 to 21 months of age at the time of vaccination showed marked increases in anti-EHV 1 antibodies at one week postvaccination (Fig. 2). These antibody titers remained elevated through nine weeks postvaccination (four weeks postrevaccination). The CMI response during this time was suppressed. Not until 13 weeks after the initial vaccination (eight weeks postrevaccination) did lymphocytes from these horses show an increased sensitivity to EHV 1. This increased sensitivity corresponded to a decline in level of anti-EHV 1 antibody. Seventeen weeks postvaccination, when the antibody titer increased more than twofold, lymphocyte transformation was again depressed. The mechanism for the production of anti-EHV 1 antibody in the six to eight month old horses may not be as fully differentiated as the CMI response. The development of high levels of antibody may, however, be dependent on prior exposure to virulent EHV 1. The younger horses may not have been exposed to EHV 1 infections, whereas the older horses may have been repeatedly

exposed. Prior exposure of older horses to virulent EHV 1 is indicated by high prevaccination anti-EHV 1 antibody titers. Lack of repeated exposure of the younger horses to virulent EHV 1 is indicated by low or no prevaccination anti-EHV 1 antibody titers.

The presence of a CMI response to EHV 1 in the six to eight month old horses and the initial absence or low CMI response to EHV 1 in the 18 to 21 month old horses may be related to the level of anti-EHV 1 antibody in the horse. Specific antibody can depress the CMI response of the host (1,2). Increased antibody synthesis leading to antibody excess may suppress the CMI response by blocking antigenic determinants on T-cells (1) or by causing the release of a factor which blocks activated T-cells (16). However, such suppression of T-cell function could occur in the absence of antibody (12). There are populations of T-cells that actively suppress the CMI response (11,22) as well as T-cells that suppress the antibody response (8, 10). The CMI response and antibody response to EHV 1 may be regulated by mechanisms associated with increased or decreased antibody synthesis involving cellular interactions among populations of amplifying and suppressor T-cells, B-cells and free and cell-associated antigen.

Pregnant horses showed significantly suppressed CMI responses but elevated antibody responses to EHV 1 following exposure to infectious EHV 1 (Table III). In contrast, stallions and nonpregnant mares responded with both elevated CMI and antibody responses. The fact that abortions in broodmares can result from dissemination of EHV 1 in the presence of high SN antibody titers to EHV 1 (3,6) suggests CMI to EHV 1 is important in preventing abortions induced by EHV 1. Suppression of CMI during pregnancy (9, 18, 23) may be due to the steroidal environment of pregnancy or lymphocyte turnover during pregnancy (17). Such suppression of the CMI response of horses to EHV 1 may make certain broodmares vulnerable to primary infection and recurring infections of EHV 1. Maximum suppression of CMI occurs in the last months of mammalian pregnancies (15) at which time 95% of abortions induced by EHV 1 occur (5). Five of the six pregnant mares in this study were nine to ten months pregnant at the time they were infected with EHV 1. The sixth mare (No. 15 in Table III), which was only six to seven months pregnant at that time, showed an increased CMI response to EHV 1. Perhaps the CMI response to EHV 1 of this single mare was not suppressed because she was not in the latter stage of a normal 11 to 12 month gestation period. None of the vaccinated broodmares in our study aborted following infection suggesting that the lymphocyte sensitivity to EHV 1 and increase in antibody response to EHV 1 was adequate to prevent dissemination of EHV 1 to the fetus.

Our results augment observations made by others that cell-mediated immunity may be important in resistance to herpesvirus infection (19). For horses to be protected against virulent EHV 1, they should have developed an adequate CMI response to EHV 1. Such protection could result from prior, subclinical EHV 1 infections or immunization with a vaccine strain of virus that induced a CMI response to EHV 1. This would be especially important for mares whose CMI response may be depressed during ensuing pregnancy and in horses less than a year of age whose anti-EHV 1 antibody response may be minimal or absent.

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