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Relationship of Antibody to Outcome in Neonatal Herpes Simplex Virus Infections

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Neutralizing antibody titers to herpes simplex virus type 1 (HSV-1) and HSV-2 were measured at birth in normal infants and uninfected infants of mothers with genital HSV infections during pregnancy and at the onset of infection in 5 infants with mild infections and 11 infants with severe infections. Thirty-eight percent of premature and 29% of term infants had neutralization titers of <1:5. High titers (≥1:40) were found in 55% of infants of mothers with primary infections during pregnancy and in 76% of infants of mothers with recurrent infections. The mean titers to HSV-1 and -2 in 5 infected infants with mild infections were 1:56 and 1:65 at the time of onset of infection, whereas the mean titers in 11 infants with severe infections were 1:11 and 1:12. Six natally exposed infants who remained asymptomatic were also studied and had a mean titer to HSV-1 of 1:85 and to HSV-2 of 1:69. Therefore, infants with high titers of transplacentally derived antibody had a more favorable outcome than infants with lower titers. Ninety-five percent of the infants of mothers with recurrent infections had a Rawls index of more than 85, suggesting that the antibody response was to HSV-2. However, low levels of antibody with this type specificity failed to protect four infants from infection with HSV-2. Augmentation of the neutralization titer to HSV-2 by the amount of complement present in cord serum was less than twofold. The study suggests that the quantity of antibody derived transplacentally affects the outcome of infection after natal exposure to herpes simplex virus. Complete neutralization of virus by antibody may occur in some infants, and prolongation of the incubation period and modification of the infection may occur in others.

Genital infections due to herpes simplex virus (HSV) are among the most common venereal diseases identified in patients from middle and upper socioeconomic groups (3, 7). The incidence of natally acquired HSV infections appears to be increasing (L. Corey, personal communication). In about half of the cases in which the baby becomes infected, maternal infection is not suspected (4, 14). Data accumulated by Nahmias et al. suggest that the risk of infection in infants exposed to HSV during vaginal delivery is less if the maternal infection is recurrent (4%) than if it is primary (50%) (13). The difference in prognosis for infants of mothers with recurrent infections as compared with those with primary infections may be related to the location and quantity of virus present and to the quantity of maternally derived antibody acquired by the infant. Adams et al. found that virus was present on the cervix in 87% of women with primary infections but in only 4% of those with recurrent infections (1). The presence of virus high in the birth canal in contrast to the exterior of the labia majora, the usual site for recurrent lesions, might also influence morbidity, since inoculation of the baby from a labial lesion might occur less often than from a cervical lesion. HSV is shed in greater quantities and for longer periods from the cervix by women with primary infections than by those with recurrent infections (1, 3).

Animal experiments have shown that the mortality of postnatally acquired HSV type 2 (HSV-2) infections can be decreased by the early administration of antibody with a high titer to HSV-2 (2, 12, 15). Although it is known that HSV can infect newborn infants in the presence of antibody, there have been few quantitative assessments of neutralizing antibody to HSV-1 and -2 in infected newborns. In this study, neutralizing antibody titers to HSV-1 and -2 were measured at the time of birth in term and premature infants and in asymptomatic infants born to mothers who had had HSV during pregnancy. In addition, neutralization titers were assessed in 6 asymptomatic exposed infants and in 16 infected infants. The quantity of antibody present was correlated with the outcome of the infection.

MATERIALS AND METHODS

Study population. Cord sera from 60 sequentially delivered, premature (birth weight < 2,000 g) infants

born in 1976 and 1977 and from 45 term infants born in 1977 were separated and stored at −20°C. Cord sera from 61 asymptomatic infants of women who had had HSV isolated from genital lesions during pregnancy and who participated in the Stanford Perinatal Herpes Simplex Project between January 1976 and December 1979 were stored in a similar manner. Twenty of these infants were born to women whose first recognized attack of HSV occurred during the pregnancy. Forty-one infants were born to mothers with a history of recurrent attacks. Seventy-one percent of these women had had recurrences in the 8th or 9th month of pregnancy.

The amount of neutralizing antibody in the cord sera of six vaginally delivered infants of mothers who had active recurrent HSV infections at the time of delivery was measured. HSV was isolated from the cervix, mons, labia, or introitus in five mothers and a Papanicolaou smear from a vesicle showed morphological changes characteristic of HSV in the sixth. The infection was first suspected at the time of delivery in four cases. In the fifth case, a Caesarian section was refused by the mother, and in the sixth a Caesarian section was not performed because the membranes had been ruptured for a prolonged period.

Sixteen infected infants delivered between April 1976 and December 1979 were also studied. None of their mothers had been followed with cultures during the pregnancy. The birth weight, type of HSV isolated, major symptoms and outcome of the infection in these infants is described in Table 1.

Measurement of neutralizing antibody to HSV. Antibody titers were measured within 6 months of the time the sera were collected in most instances. Samples from normal premature and term infants were tested simultaneously. Sequential samples from infected infants were tested on the same day. The same pools of HSV-1 and -2 were used throughout the study.

By using microtiter U plates (Nunclon), 0.025 ml of each serial dilution of serum was incubated with an equal volume of 100 50% tissue culture infective doses of HSV-1 (MacIntyre; ATCC VR 539) or HSV-2 (MS; VR 540) in replicates of six at 37°C for 1 h. Human foreskin fibroblasts (200,000 cells per ml) were then added to each well in a volume of 0.05 ml. After 4 days or when the back titration read 100 50% tissue culture infective doses ± 0.5 $\log_{10}$, each well was scored as to whether cytopathic effect was present or absent. The 50% endpoint for the six replicates was calculated by the Karber method (10). The Rawls index was calculated by the formula: ($\log_{10}$ antibody titer to HSV-2/ $\log_{10}$ antibody titer to HSV-1) × 100 (16).

Typing of HSV strains. Strain typing was done by restriction endonuclease analysis in the laboratory of Stephen Sacks and Thomas Merigan.

Measurement of hemolytic complement levels. Cord blood samples were collected at the time of delivery, allowed to retract for 60 min at room temperature, centrifuged, and stored at −70°C. Fifty percent hemolytic complement levels were determined by the method of Meyer as outlined by Ruddy and Austen (17). Samples which had been stored in such a way as to preserve native complement were tested before and after heat inactivation at 56°C for 1 h.

Lymphocyte transformation. Peripheral blood mononuclear cells were separated on a Ficoll-Hypaque gradient. The cells were then cultured in McCoy media and 30% human sera in microtiter wells containing 3 × 10^5 cells and 1:3, 1:10, or 1:30 dilution of inactivated HSV-2 antigen. After 4 days of incubation at 37°C,

TABLE 1. *Clinical findings in infants infected with HSV*

HSV infection	Case	Wt (g)	HSV type	Major manifestations of infection	Follow-up
Mild	A	4,200	1	Single lesion, vocal cords	Surviving at age 2.25 yr; no sequelae
	B	1,134	2	Scalp vesicles, asymptomatic meningitis	Surviving at age 4.5 yr; speech delay
	C	2,880	2	Skin lesions only	Surviving at age 3.25 yr; no sequelae
	D	2,500	ND[a]	Skin lesions only	Surviving at age 10 mo; no sequelae
	E	2,190	2	Skin lesions only	Surviving; no follow-up after 3 months
Severe	F[b]	2,922	1	Disseminated infection	Surviving at age 2.5 yr; hemiparesis; bilateral retinal detachment; blind
	G	1,210	2	Disseminated infection	Expired at age 5 weeks.
	H	2,895	ND	Encephalitis; no skin lesions	Expired at age 6 weeks.
	I	2,863	ND	Encephalitis; no skin lesions	Surviving at age 2 yr; severe neurological sequelae
	J	3,500	1	Disseminated infection	Expired at age 11 days
	K	3,800	2	Encephalitis; no skin lesions	Expired at age 7 weeks
	L	3,300	2	Encephalitis; no skin lesions	Surviving at age 12 mo; severe neurological sequelae.
	M	2,950	2	Encephalitis; no skin lesions	Expired at age 6 mo
	N	3,500	ND	Encephalitis; no skin lesions	Expired at age 6 wk
	O	2,800	ND	Encephalitis; pneumonia, skin lesions	Expired at age 15 days
	P[b]	3,040	2	Encephalitis; no skin lesions	Surviving at age 3 mo; severe neurological sequelae.

[a] ND, Not determined.
[b] Treated with adenine arabinoside.

tritiated thymidine was added. Cultures were harvested on day 5 with a multiple-sample automated harvester, and the incorporation of tritiated thymidine was measured with a liquid scintillation counter. A threefold increase in counts per minute in the antigen-stimulated wells as compared with the control wells was considered significant. Such increases have been previously shown to correlate with serological evidence of immunity (8).

The antigen was prepared from monolayers of infected HeLa cells showing 75 to 90% cytopathic effect. Cells were harvested and subjected to six freeze-thaw cycles. The antigen was clarified by centrifugation at 1,000 × *g* for 10 min, inactivated at 56°C for 2 h, and stored at −70°C until use. A control antigen was prepared in the same manner from uninfected HeLa cells.

RESULTS

The prevalence of normal infants with neutralization titers of <1:5 to both HSV-1 and HSV-2 was 38% in premature (<2,000 g) and 29% in term infants, as shown in Table 2. Of 20 infants born to women who had their first clinical attack of genital herpes during pregnancy, 10% had titers of <1:5 to HSV-1 and 5% had titers of <1:5 to HSV-2. All of the infants of mothers with recurrent infections had detectable antibody to both HSV-1 and -2.

TABLE 2. *Neutralization titers to HSV-1 and HSV-2 in cord sera of normal infants and infants of mothers with HSV infections during pregnancy*

Virus neutralization titer	% of infants			
	Normal		Maternal HSV	
	Premature (60)[a]	Term (45)	Primary (20)	Recurrent (41)
HSV-1				
<5	38	29	10	0
5–9	5	2	0	2
10–19	13	7	20	10
20–39	22	11	30	33
40–79	15	13	35	33
80–159	5	13	5	14
160–319	2	11	0	7
≥320	0	13	0	0
≥1:40	22	50	40	54
HSV-2				
>5	38	29	5	0
5–9	7	7	15	2
10–19	25	4	10	7
20–39	18	13	15	14
40–79	8	18	30	38
80–159	3	7	25	19
160–319	0	11	0	17
≥320	0	11	0	2
≥1:40	11	47	55	76

[a] Numbers in parentheses indicate number of infants.

High neutralization titers to HSV-1 (≥1:40) were found as frequently in normal term infants as in infants of women with primary or recurrent HSV infections during pregnancy ($P > 0.1$). Neutralization titers of ≥1:40 to HSV-1 were found in 50% of term infants, 40% of infants of mothers with primary infections, and 54% of infants of mothers with recurrent infections.

Infants of mothers with recurrent infections had high titers (≥1:40) to HSV-2 more often than infants of mothers with primary infection ($P = 0.06$) or normal term infants ($P = 0.03$). Titers of ≥1:40 to HSV-2 were found in 76% of infants of mothers with recurrent infections, 55% of infants of mothers with primary infections, 47% of term infants, and 11% of premature infants. Thirty of forty-seven infants (64%) of mothers with HSV during pregnancy who had titers of ≥1:40 to HSV-2 also had titers of ≥1:40 to HSV-1. Only 13 (28%) of the 47 infants had an HSV-2 titer which was twofold higher than the HSV-1 titer.

The Rawls index (16) was more than 85 in all but two (4.9%) of the infants of mothers with recurrent infections, which suggests that the antibody response was induced by HSV-2 in most cases. The mean Rawls indexes and standard deviations were 112 ± 17.40 in infants of mothers with recurrent infections, 103 ± 24.31 in infants of mothers with primary infections, and 96 ± 18.74 in normal term infants.

Since complement may potentiate the efficiency of neutralization of HSV (18), sera from seropositive newborns and adults were tested in the presence and absence of native complement. The mean 50% hemolytic complement level for 17 cord sera was 18 U as compared with a mean of 34 U for the adult sera. A modest increase in neutralizing antibody titer occurred when newborn sera were tested in the presence of native complement. The mean titer of 14 seropositive sera was 1:41 in the absence of complement and 1:52 in the presence of native complement. The degree of augmentation of neutralization by native adult complement was similar to that seen with native newborn complement despite the fact that the adult sera contained approximately two times as much complement as the cord sera. The mean neutralization titer in the adult sera was 1:46 in the absence of complement and 1:56 in the presence of complement.

Various immunological parameters were studied in 16 infected infants, and the results were correlated with outcome of the infection. The mean titers to HSV-1 and HSV-2 were 1:56 and 1:65 in those with mild infections as compared with 1:11 and 1:12 in those with severe infections.

TABLE 3. *Neutralizing antibody titers to HSV-1 and HSV-2 in infants with mild and severe HSV infections at onset and in natally exposed, asymptomatic infants at birth*

HSV infection	Case	Neutralization Titers	
		HSV-1	HSV-2
Mild	A	1:6	1:6
	B	1:115	1:115
	C	ND[a]	1:34
	D	1:47	1:100
	E	1:56	1:71
Severe	F	1:4	<1:5
	G	1:20	1:16
	H	1:7	<1:5
	I	1:10	1:12
	J	<1:5	<1:5
	K	1:16	1:25
	L	1:20	1:18
	M	<1:5	<1:5
	N	1:13	1:25
	O	<1:5	<1:5
	P	1:20	1:18
Natally exposed asymptomatic	Q	1:71	1:50
	R	1:40	1:71
	S	1:141	1:50
	T	1:79	1:89
	U	1:35	1:63
	V	1:141	1:89

[a] ND, Not determined.

As shown in Table 3, 5 of the 11 infants with severe HSV infections had HSV-2 titers of <1:5 near onset and the remainder had titers of <1:25. In contrast, four of five infants with mild infections had titers of >1:34. The discrepancy between the HSV titers of mother and infant in case A, an infant of a mother with a primary infection in the first month of pregnancy, is shown in Fig. 1 and remains unexplained.

High antibody titers were found in all of the six asymptomatic vaginally delivered infants of mothers with active infections at the time of delivery, as shown in Table 3. The mean titers to HSV-1 and -2 in these infants were 1:85 and 1:69, respectively.

The antibody titers of the five infants with mild infections are shown in relationship to the onset of infection and the mother's antibody titer in Fig. 1. The titers for 8 of the 11 infants with severe infections are shown in Fig. 2. The titers waned for at least 4 weeks after onset of the infection in five of eight infants tested sequentially. Infants F, H, and P developed increases in antibody titer within 3 weeks of the onset of infection. Infants F and P were also the only two infants who received adenine arabinoside.

Waning transplacentally acquired antibody might prolong the incubation period and explain the delayed onset of symptoms in infants inoculated at birth. Antibody titers were measured at birth and at 3 to 5 weeks of age in eight asymptomatic infants of mothers who had had genital HSV infections during pregnancy and who had not had blood drawn for any other purpose. As shown in Fig. 3, the slope of the decay curve was variable. Four of five infants with titers of ≥1:40 to HSV-2 at birth had titers of <1:40 by 3 to 5 weeks of age.

Lymphocyte transformation to HSV was tested sequentially in five of nine infants with severe infections who survived 7 or more days after onset. The results in three of the five infants are shown in Fig. 2. One infant developed a positive response between 6 and 14 days after onset of illness, and another developed a positive response between days 13 and 22 after onset. Lymphocyte transformation was not demonstrable in the remaining three infants for 3 weeks or longer after onset. In contrast, the mean phytohemagglutinin stimulation index was 29.1 in 13 assays in which the HSV-2 stimulation index was less than 3. As shown in Fig. 1, lymphocyte transformation was also assessed in two infants who did well and was negative 6 days after onset in case E and positive 11 days after onset in case D.

Although infants with high neutralization titers appeared to have a better prognosis than infants with low titers, birth weight did not appear to correlate with outcome. As shown in Table 1, three of the five infants with mild infections were premature and had birth weights of 1,134, 2,190, and 2,500 g. Ten of eleven infants with severe disease weighed more than 2,800 g. Of the 11 infants with severe disease, 6 expired at less than 8 weeks of age and 1 expired at home at 6 months of age from the sequelae of catastrophic central nervous system damage.

DISCUSSION

Antibody to HSV has been shown to decrease the mortality rate in neonatal murine HSV infections if given in sufficient quantities soon after inoculation with the virus (2, 12, 15). Oakes and Rosemond-Hornbeak showed that administration of antibody up to 24 h after inoculation prevented spread of HSV from the footpads of mice (15). The circulating neutralizing antibody titer in the mice 3 days after the administration of antiserum was 1:64. Passive antibody did not, however, protect irradiated mice, indicating that participation of a radiation-sensitive cell may be necessary for antibody to interrupt cell-to-cell spread of HSV (11). Shore et al. (20) and Kohl et al. (9) have shown that nonimmune newborn

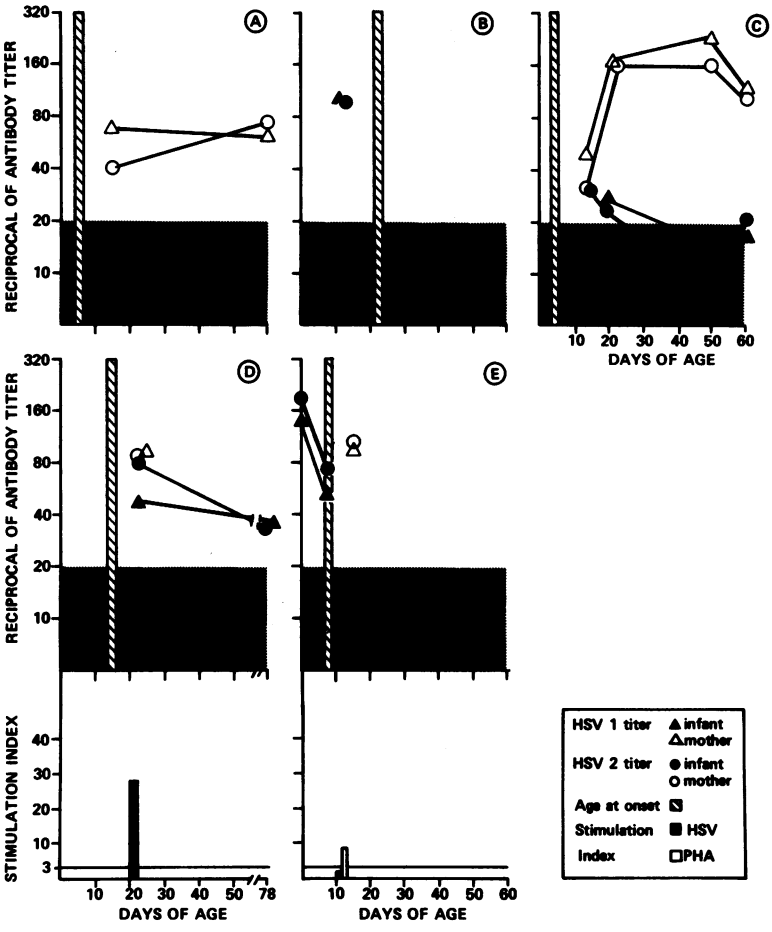

FIG. 1. *Neutralizing antibody titers and lymphocyte stimulation assays in five infants with mild HSV infections.*

lymphocytes and macrophages can mediate antibody-dependent cellular cytotoxicity as assessed by the release of ^{51}Cr from labeled Chang liver cells. Shimizu et al. have shown that nonimmune mouse peripheral blood lymphocytes and antibody can restrict cell-to-cell spread in monolayers as judged by plaque size, immunofluorescent foci, and production of infective virus (19).

The results of this study suggest that antibody may also influence the course of human neonatal infections. Complete neutralization of virus may have occurred in the six natally exposed, asymptomatic infants of mothers who were shedding virus at delivery. These infants had mean serum neutralization titers to HSV-1 and -2 of 1:85 and 1:69. Maternal antibody in blood and secretions in the birth canal may also have helped protect these infants from infection. The five infants with mild HSV infections had mean titers to HSV-1 and -2 which were fivefold (1:56 and 1:65) higher than the titers of infants with severe infections (1:11 and 1:12). Neutralization titers of $\geq$1:40 were, therefore, associated with a more favorable outcome than were lower titers. Titers of $\geq$1:40 to HSV-2 were found in 55% of infants born to mothers with primary HSV infections and in 76% of infants of mothers with recurrent infections. Therefore, one out of every two infants of mothers with primary infections and one out of every four infants of mothers with recurrent infections had antibody titers which were lower than those of natally exposed infants who remained asymptomatic and infected infants with mild illnesses.

Ninety-five percent of the infants of mothers with recurrent HSV infections during pregnancy had Rawls indexes of more than 85, which suggests that the antibody response was to HSV-2. However, 64% of the infants of mothers with

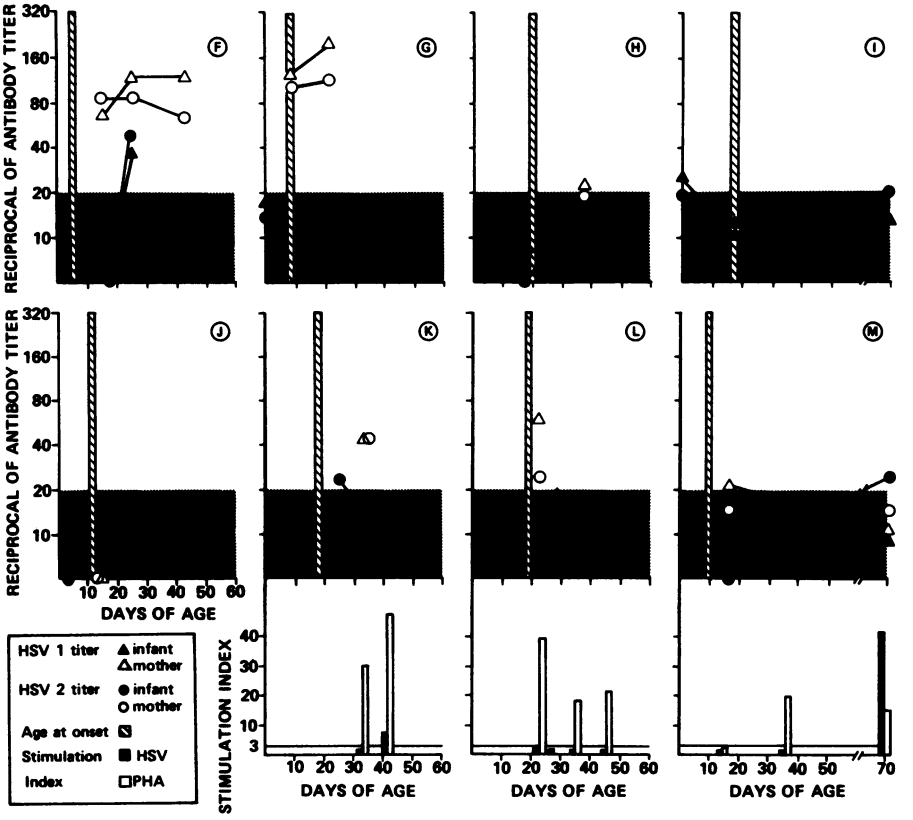

FIG. 2. *Neutralizing antibody titers and lymphocyte stimulation assays in eight infants with severe HSV infections.*

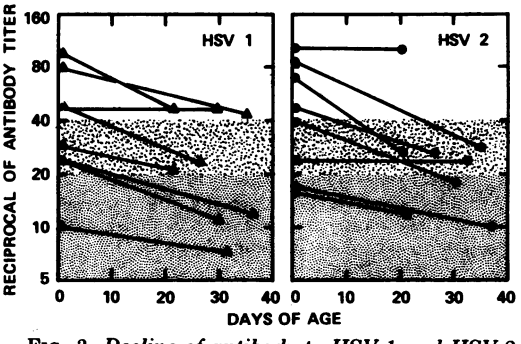

FIG. 3. *Decline of antibody to HSV-1 and HSV-2 over the first month of life in normal seropositive infants.*

recurrent disease who had antibody titers of ≥1:40 to HSV-2 also had antibody titers of ≥1:40 to HSV-1. All of the asymptomatic vaginally delivered infants of mothers with active infections had antibody titers of >1:35 to HSV-1 as well as titers of ≥1:40 to HSV-2, as did all of the infants with mild infections. Analysis of the glycoproteins of HSV-1 and -2 suggests that an antigenically identical glycoprotein is found in both HSV-1 and -2. Monoclonal antibody to this glycoprotein can protect mice against both viruses (R. D. Dix, L. Pereira, and J. D. Baringer, Abstr. Int. Conf. Human Herpesviruses, Atlanta, Ga. 1980). The fact that low levels of type 2 antibody (Rawls indexes, 92 to 116) did not protect infants G, K, L, and P from severe infection with HSV-2 suggests that the quantity of transplacentally acquired neutralizing antibody may correlate more closely with prognosis than type of antibody.

Native complement increased the neutralization titer of the transplacentally derived antibody in the infant's serum less than 2-fold as compared with the 2- to 16-fold increases reported in vitro (18). Despite the fact that the newborn infants had lower complement levels than the adults studied, the degree of augmentation of the neutralization titers by the native complement in adult and newborn serum was the same.

The immune response to HSV developed slowly in the infected infants in this study. An-

tibody titers declined for 4 weeks in five of the eight (63%) infected infants studied sequentially. Five infants with severe infections had lymphocyte stimulation assays performed. Only one had a positive response within 2 weeks of onset of the infection. Corey et al. have shown that the magnitude of the peak HSV stimulation index correlates inversely with the duration of shedding of HSV in adults with primary genital infections (6).

If the correlation between the infant's antibody titer and prognosis is confirmed, it may be possible to restrict delivery by Caesarian section to women with primary infections and to those with active recurrent lesions and low antibody titers. It will be necessary, however, to determine whether the antibody titers of the infants in the latter group can be predicted from the mother's titer in the third trimester. The presence of the mother's antibody in blood and secretions in the birth canal at the time of delivery may also be important.

The infected infants we studied who did poorly had low antibody titers at the onset of infection as well as delayed antibody production. Four of five uninfected infants who had antibody titers of $\geq$1:40 at birth had titers of $<$1:40 by 3 to 5 weeks of age. Further studies should be done to determine whether the administration of antibody to HSV would benefit newborn infants exposed to or infected with HSV. In vitro assays and animal experiments show that antibody, in cooperation with nonimmune lymphocytes and macrophages, can restrict the cell-to-cell spread of HSV. There is already evidence in animal models that antibody and antiviral agents may potentiate one another in controlling the spread of HSV (5). The influence of antibody on outcome should be assessed as an independent variable in future trials of experimental antiviral agents for the treatment of neonatal HSV infections.

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