PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 291–297 (1982)

Determinants of Protection by Human Immune Globulin against Experimental Herpes Neonatorum[1] (41433)

J. A. GEORGIADES,*[,2] J. MONTGOMERY,* T. K. HUGHES,* D. JENSEN,† AND S. BARON*

*Department of Microbiology, University of Texas Medical Branch, Galveston, Texas 77550, and †Pathology Department, Brooke Army Medical Center, San Antonio, Texas 78234

Abstract. There is no established prophylaxis of Herpes neonatorum. In experimental, newborn animals protection can be achieved by sufficient passive antibody given shortly after infection. Animal model data was sought to estimate a realistic and practical dose of human immune globulin for prophylaxis of newborn humans at risk. We used a neonatal mouse model and type II herpes simplex virus (HSV) to evaluate factors which govern the efficiency of antibody prophylaxis. Antibodies prepared in mice, rabbits, or man were equally protective. Timing experiments showed that these antibodies gradually lose their protective effect when administered more than 12 hr after infection. The first dose of multiple doses of antibody is the most crucial for protection. Intraperitoneal or subcutaneous injections of human immune globulin were equally effective. About 65 units of human anti-HSV antibody in 1-g newborn mice was the minimum dose which resulted in maximum protection. Comparison of this minimum protective dose with levels of neutralizing antibody in commercial immune globulin indicated that approximately 100 ml would be needed to achieve similar protective serum antibody levels in man. Six percent of potential blood donors were determined to have sufficient HSV antibody titers ($\geq$1000 units) to prepare hyperimmune globulin at least 10 times more potent than commercially available immune globulin. Only 10 ml or less of such hyperimmune globulin is estimated to be protective. This reduced volume of hyperimmune globulin is easily injected into the loose subcutaneous tissue in the backs of newborn children.

Herpes neonatorum often is a generalized herpes simplex virus (HSV) infection of newborn infants, which is almost always highly fatal or debilitating. The infection most often occurs during the passage of the baby through the birth canal of a mother undergoing active infection of the cervix or vagina, although less frequently infections may occur intrauterally or postnatally. A small group (approximately 14%) of recently infected mothers have normal offspring (1–5). The mechanisms involved in protection are only incompletely understood. Although cell-mediated immunity is thought to play an important role in herpesvirus infections, adequate levels of antibody are protective in animal model infections (6–18). In man placentally transferred antibodies may be one of the reasons for natural protection of some infants against herpes neonatorum (19).

Early attempts at protection against herpes neonatorum in one human case (14) and many animal trials (17, 18, 20) by administration of commercially available immune globulin were unsuccessful. It was believed, therefore, that the antibody response did not play an important role in protection. However, the reason for lack of protection may have been the low concentrations of antibodies in these passively administered sera. We and others have shown, for example, that hyperimmune antisera to HSV will protect newborn mice against a lethal dose of HSV given subcutaneously, intranasally, and intracerebrally (15, 16, 21, 22). We now extend the studies in the mouse model to: (a) examine the dose variables which govern the effectiveness of

[1] Supported by the March of Dimes National Foundation Grant 1-515.

[2] To whom reprint requests should be addressed.

0037-9727/82/070291-07$01.00/0

neutralizing antibody during postexposure prophylaxis of experimental HSV infection, (b) determine the effectiveness of human antibodies in various potencies in protection against experimental herpes neonatorum, and (c) determine whether human antibodies are protective against different antigenic types and strains of HSV. Finally, from the estimate quantity of passive antibody required to achieve protection levels of serum antibody in human newborns, we determined the percentage of blood donors in the population that have sufficient antibody for production of hyperimmune globulin.

The data show that antibody dose and timing strongly influence the protective effect against herpes neonatorum. A projection of the quantity of human antibody required to prevent the human disease indicated that ordinary immunoglobulin is impractical and that approximately 6% of surveyed donors have sufficient antibody to HSV type II (HSV II) for use in production of suitable hyperimmune globulin.

Materials and Methods. *Tissue culture and media.* Human foreskin fibroblast cells (HFS-4) were obtained from Dr. J. Vilcek, New York University Medical School. Cells in flasks or microtiter plates were grown in Eagle's minimum essential medium (MEM), supplemented with 10% heat-inactivated fetal calf serum (FCS), and 100 units of penicillin G/ml and 1 μg of streptomycin/ml. Serum was reduced to 2% for experiments.

Viruses. HSV type II MS strain was used for most studies. Where indicated, we also used the following strains: HSV II, 75-730; HSV II, 76-632; HSV II, Greenwood; and HSV I KET. These viruses were obtained through the courtesy of Dr. A. J. Nahmias, Emory University. Plaque titers of viruses were determined using HEP-2 cells in a microtiter plaque assay (23). Plaques were enumerated microscopically using two to four replicates and repeated assays. Differences of 50% are statistically significant ($P < 0.05$).

Antibody to HSV type II. Two types of antibodies against HSV type II were used: rabbit antibody as hyperimmune serum, which was previously described (21), and human antibody, as commercial human immune globulin. Twenty-six batches of commercially prepared immune globulin preparations were tested for the level of antibodies against HSV type II. Four immune globulin preparations were provided by Hyland Laboratories, 10 from Merck, Sharp and Dohme Company, 2 from Savage Company, and 10 from Wyeth Pharmaceuticals. Before use in antibody protection experiments, all batches of immune globulin were assayed *in vitro* for plaque neutralization of NSV II.

Five hundred fifty-six serum samples were obtained from blood donors in Galveston County, Texas. They were collected with the help of Dr. E. Patten of the University of Texas Medical Branch Blood Bank service.

Neutralization tests. Serial twofold dilutions of sera were mixed with equal volumes of virus (20–30 PFU/0.1 ml) and incubated at 37° for 1 hr. Two-tenths milliliter of each mixture was placed in duplicate or quadruplicate on HEP-2 cells grown in microtiter II plates and incubated overnight. One unit of antibody is calculated as the reciprocal of the highest dilution of antibody and reduces the number of plaques by 50%. Since two replicas were used, 50% reduction means discrimination of the average of 50 control PFU to 25 endpoint PFU. Antibody titer differences of more than twofold are statistically significant ($P < 0.05$).

Mice protection tests. Two- to four-day-old litters of randomly bred Swiss–Webster mice were used as described previously (21). Briefly, the newborn mice were inoculated subcutaneously with a LD_{75} dose of HSV suspended in 0.05 ml of medium. At various times after inoculation, groups of inoculated and control mice received different amounts of immunoglobulin in 0.05 ml volumes. Sites of injection of immunoglobulin are specified for each experiment and were always different from the site of virus injection. Inoculated mice were observed for two weeks for signs of illness and for death. He accepted only experiments where 75–90% mice died in control group. Results were evaluated by percentage pro-

tection, which was calculated as

$$\% \text{ protection} = \frac{\% \text{ mortality of controls} - \% \text{ mortality of treated}}{\% \text{ mortality of controls}}$$

The results are presented as percentage protection. The number of mice that died in each experiment can be calculated from the percentage protection, number of mice in each group, and the percentage mortality in the control groups.

Results. We first attempted to establish the relationship between the time of antibody (immunoglobulin) administration relative to virus injection and the degree of protection (Table I). Administration of antibody (160 units) 1–12 hr after HSV inoculation resulted in significant protection (64 to 80% protection). Injection of antibody 24 hr postinoculation showed only 30% protection. Consequently to achieve maximum protection in subsequent experiments, we administered antibody 1 hr after virus.

We compared the protective effect of immune globulin injected in a site of rapid absorption (intraperitoneal) with injection in a site of slower absorption (subcutaneous). No significant difference was observed between intraperitoneal versus subcutaneous administration of antibody (Table II). About 64 units of human anti-HSV antibody injected by either route resulted in maximum protection. Since the two routes of antibody administration gave equal protection, the results from both series of experiments were pooled for construction of a cumulative dose–response curve (Fig. 1). A careful comparison of the protective titers of rabbit (21) and human antibodies demonstrated that they were equally effective (data not shown).

Protection by single and multiple doses of antibodies are shown in Table III. The total amount of antibody administered by multiple injections were designed either to equal the amount administered by single injection or to double or triple the single injection amount. The data obtained indicate that the first dose of antibody is the most crucial for protection. Furthermore, protection increased with higher initial doses to a maximum value of up to 90% survival, after which further increasing the antibody dose had little further protective effect.

Protection against both types of HSV and their strains is a requisite for an antibody preparation to be used to prevent the human disease. Neutralization of different antigenic types and strains of HSV by commercially available human immune

TABLE I. RELATIONSHIP BETWEEN TIME OF ANTIBODY ADMINISTRATION AND PERCENTAGE PROTECTION[a]

Expt no.	Hour of treatment after infection	Number of mice	Antibody (units)	Protection (%)
76	1	21	160	64*
112	1	30	160	80*
112	12	31	160	72*
76	24	20	160	30**
112	30	42	160	0
115	36	61	160	0
76	48	19	160	22
115	54	68	160	12
112	60	43	160	0
112	72	43	160	0
115	72	69	160	0

[a] Human immune globulin was injected intraperitoneally.
* $P < 0.005$.
** $P < 0.05$.

TABLE II. COMPARISON OF PROTECTION USING INTRAPERITONEAL AND SUBCUTANEOUS ADMINISTRATION OF ANTIBODY

Route	Number of mice	No. expts	Units of antibody	Percentage protection ± SD
IP	74	(4)	240	61 ± 20
SQ	18	(1)	240	72
IP	73	(3)	160	75 ± 10
SQ	85	(3)	160	66 ± 16
IP	51	(2)	100	86 ± 20
SQ	136	(4)	100	68 ± 10
IP	48	(1)	48	79
SQ	53	(1)	48	73
IP	107	(6)	24	44 ± 18
SQ	55	(1)	24	52
IP	100	(3)	12	14 ± 13
SQ	162	(4)	12	34 ± 23

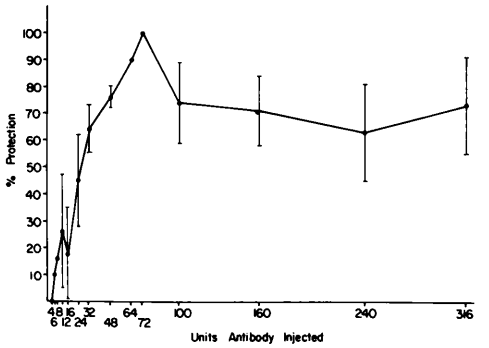

FIG. 1. Relationship between the quantity of human antibody administered and percentage protection. Combined results from Tables II–IV. Vertical lines indicate standard deviation.

globulin is shown in Table IV. The data indicate that antibodies in human immune globulin preparations protect mice equally well against several strains of HSV types I and II as well as their substrains.

To estimate the feasibility of applying commercially prepared human immune globulin to humans, plaque neutralization titers were determined for 26 batches. The neutralizing titers were close to each other and averaged 3.35 ± 0.18 log units/ml. As shown above, 64 units of human anti-HSV antibodies afforded maximal protection to 1-g newborn mice. Since protection by antibody is correlated with its concentration in plasma, and since plasma comprises approximately the same percentage of body weight in mice and humans (25), the same amount of antibody per gram is needed to protect a newborn baby. Thus a baby weighing about 3500 g would need 224,000 units of antibody. The average commercial immune globulin has 2238 units/ml. Therefore, approximately 100 ml would be necessary to achieve the protective levels of antibody in a human newborn. This volume is not practical for immunoprophylaxis and preparations of higher titer are needed. Thus, screening of human donors to identify those with high titers of HSV antibody was done. The distribution of antibodies against HSV type II among donor populations originating from Galveston County, Texas, is shown in Fig. 2. The results show that approximately 6% of the donors had antibody titers ≥1000 units/ml and would be suitable for production of the hyperimmune globulin that is estimated to be protective in human newborns when given in a volume of 10 ml or less.

Discussion. Administration of sufficient amounts of human antibodies as immune globulin protected HSV II-infected neonatal mice against mortality. These results are in good agreement with our previous findings with rabbit anti-HSV antibody (21). The maximum protection by antibody averages 70%. This estimation may be too conservative since there is a 10–20% mortality in our mouse colony among uninfected newborn mice. Antibodies prepared

TABLE III. COMPARISON OF PROTECTION WITH SINGLE AND MULTIPLE DOSAGES OF ANTIBODY

Number of mice	No. expts	Antibody units/dose	Number of doses (daily)	Total antibody	Percentage protection ± SD
115	(5)	316 u	1	316	73 ± 18
85	(3)	158 u	2	316	66 ± 16
92	(3)	105 u	3	316	64 ± 5
44	(2)	100 u	1	100	74 ± 15
43	(1)	32 u	3	96	54
17	(1)	64 u	1	64	90
40	(1)	32 u	2	64	66
126	(3)	32 u	1	32	64 ± 9
91	(3)	16 u	2	32	40 ± 9
19	(1)	10 u	3	30	10
101	(2)	48 u	1	48	76 ± 4
17	(1)	24 u	2	48	22
58	(2)	16 u	3	48	39 ± 55

TABLE IV. PROTECTION AGAINST VARIOUS STRAINS OF HERPESVIRUS TYPES I AND II BY HUMAN IMMUNE GLOBULIN

Expt no.	Virus, strain	Number of mice	Percentage protection		
			16 units of antibody[a]	32 units of antibody	64 units of antibody
H-133	HVH II, 75-730	88	62	79	74
H-138	HVH II, 76-532	94	71	85	59
H-135	HVH I, Greenwood	70	44	91	100
H-117	HVH I, KET	74	77	70	—
H-108	HVH II, MS	110	48	52	82

[a] Antibody titers were determined against HSV II, MS strain.

in mice (2), rabbits, or man seem equally protective.

The protection by antibodies is strongly influenced by total dose and time of administration. Optimum protection of newborn mice occurred when a minimum of approximately 64 units were given 1–12 hr after infection at 24 hr; lower but significant protection occurred. After 24 hr little or no protection occurred which is consistent with previous reports (22, 24). In man the time available for effective postexposure prophylaxis may be longer due to the longer incubation period. A conservative estimate for the human situation indicates that hyperimmune globulin should be available for treatment within 12 hr after delivery. Such a putative distribution scheme could readily be organized for urban areas but would be more difficult to arrange for rural areas.

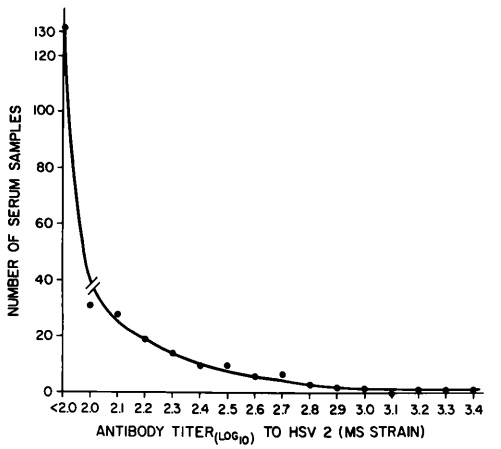

FIG. 2. Distribution of antibody levels to HSV II in human sera from Galveston County, Texas.

The data obtained from comparison of a single antibody dose with multiple doses indicated that the first dose was the most critical in prevention of HSV-induced mortality. Also, the intraperitoneal and subcutaneous routes of antibody administration seem indistinguishable for protection against HSV.

Passive immunization with commercial human immune globulin did not protect infected infants (14). One explanation is that the protective levels of plasma antibody are not provided by ordinary immune globulin. Our comparison of the titer of neutralizing antibodies in 26 batches of commercial immune globulin showed almost identical levels of HSV type II antibodies (3.35 ± 0.4 log units/ml). As estimated under Results, approximately 100 ml of immune globulin may be required for effective postexposure prophylaxis of infants. Commercially available immune globulin, therefore, is not practical for prophylaxis of herpes neonatorum. Alleviation of this problem would seem to lie in the production of higher titered preparations of immune globulin. A survey of the blood donors recruited from the population of Galveston County, Texas, showed that approximately 6% had antibody levels of $\geq$1000 U/ml and would be suitable for production of the hyperimmune globulin. Calculations indicate that enough hyperimmune globulin for each 1000 newborns at risk can be prepared from 500 selected donors. Our experience indicates that the effort involved in selection of a large number of suitable donors and their monitoring for repeated bleeding is feasible even for a single laboratory. Based on ex-

pected serum antibody levels, such hyperimmune globulin should be protective in human newborns when given in a volume at or below 10 ml. We believe that 10 ml is a realistic volume to administer subcutaneously in the back since we have injected 30 ml of immune globulin into a 3-pound newborn without untoward effects.

There are many uncertainties in the extrapolations from the mouse model to man. To compensate, our estimations have been made conservatively to underestimate protection in man. For example, we used a 75% LD_{50} challenge dose of virus in our animal model. Data not presented showed equal protection with a 100% LD_{50}. In comparison, the lethality in human newborns is only about 35%. Since more antibody is needed to protect against higher virus challenge doses, our estimate of the amount of antibody needed in man is probably high. The usefulness of this treatment in man may be partially limited because the newborn may be infected *in utero* or postnatally. Also perhaps only one-third of infected mothers might have a recognized lesion. Improved diagnostic methods may overcome these partial limitations.

The present delineation of many of the requirements for protection by passive antibody indicates that hyperimmune globulin should be produced and tested in humans. Prior to studies in man, validating experiments in subhuman primates are indicated.

1. Nahmias AJ, Alford CA, Korones SB. Infection of the newborn with herpesvirus hominis. Advan Pediatr 17:185–226, 1970.
2. Hanshaw JB. Herpesvirus hominis infections in the fetus and the newborn. Amer J Dis Childh 126:546–555, 1973.
3. Nahmias AJ, Roizman B. Infection with herpes-simplex viruses 1 and 2. N Engl J Med 289:667–679, 719–725, 781–789, 1973.
4. Hare MJ. Neonatal encephalitis following maternal genital herpesvirus infection. Proc R Soc Med 67:15–16, 1974
5. Nahmias AJ, Josey WE, Naib ZM. Perinatal risk associated with maternal genital herpes simplex virus infection. Amer J Obstet Gynecol 110(6): 825–827, 1971.
6. Berry GP, Slavia H. Studies on herpetic infection in mice. J Exp Med 78:305–320, 1943.
7. Cheever FS, Daikos G. Studies on the protective effect of gamma globulin against herpes simplex infections in mice. J Immunol 65:135–141, 1950.
8. Tokumaru T. The protective effect of different immunoglobulins against herpetic encephalitis and skin infection in guinea pigs. Arch Gesamte virusforsch 22:332–398, 1967.
9. Gruia M, Mutin A, Gristea AL, Sundan C, Gologan R, Lupu R, Wegsler P. The action of human immunoglobulins in experimental herpes infection in the newborn rat. Rev Roum Virol 10:47–58, 1973.
10. Akerfeldt S, Lofberg E, Fuchs G. Antiviral effect of human gamma globulin in mice. Comparison between the efficacy of local and systemic administration. Biochem Pharmacol 22:2911–2917, 1973.
11. Baker MB, Larson CL, Ushijima RN, Anderson FD. Resistance of female mice to vaginal infection induced by herpesvirus hominis type 2: Effects of immunization with mycobacterium bovis, intravenous injection of specific herpesvirus hominis type 2 antiserum, and a combination of these procedures. Infect Immun 10:1230–1234, 1974.
12. Luyet F, Samara D, Soneji A, Marks MI. Passive immunization in experimental herpesvirus hominis infection of newborn mice. Infect Immun 12:1258–1261, 1975.
13. Worthington MG, Williams J, Baron S, Couliffe AM. Thymus dependent antibody formation to herpesvirus hominis. Immunol Allergy; Parasitol Infect Dis 3:370, 1975.
14. Wheeler CE Jr, Huffines WD. Primary disseminated herpes simplex of the newborn. J Amer Med Assoc 194:455–460, 1965.
15. Evans CA, Slavin HB, Berry GP. Studies on herpetic infections in mice; affect of specific antibodies on progression of virus within nervous systems of young mice. J Exp Med 84:429–447, 1946.
16. Bedson SP, Crowford GJ. Immunity in experimental herpes. J Exp Pathol 8:138–147, 1927.
17. Allison AC. Summary and discussion of future applications of vaccination and immunotherapy. Monogr Allergy 9:259, 1975.
18. Oakes JE. Role for cell-mediated immunity in the resistance of mice to subcutaneous herpes simplex virus infection. Infect Immun 12:166–172, 1975.
19. Yeager AS, Arvin AM. Relationship of antibody to outcome in neonatal herpes simplex virus infections. Infect Immun 29:532–538, 1980.
20. Koyama H, Kossahara S. Induction of herpes simplex virus immunity in newborn mice. Infect Immun 12:1472–1474, 1975.
21. Baron S, Worthington MG, Williams J, Gaines JW. Postexposure serum prophylaxis of neonatal herpes simplex virus infection of mice. Nature (London) 261:5560, 505–506, 1976.

22. Cho CT, Feng KK, Bramacupta N. Synergistic antiviral effects of adenine arabinoside and humoral antibodies in experimental encephalitis due to herpesvirus hominis. J Infect Dis 133(2):157–167, 1976.
23. Randhawa AS, Stanton GJ, Green JA, Baron S. Variables affecting viral plaque formation in microculture plaque assays using homologous antibody in liquid overlay. J Clin Microbiol 5:535–542, 1977.
24. Luyet F, Samr D, Soneji A, Marks MI. Passive immunization in experimental herpesvirus hominis infection of newborn mice. Infect Immun 12:1258–1261, 1975.
25. Wish L, Furth J, Storey RH. Direct determination of plasma, cell and organ-blood volumes in normal and hypervolemic mice. Proc Soc Exp Biol Med 74:644–648, 1950.

Received January 22, 1981. P.S.E.B.M. 1982, Vol. 170.