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Production of Herpes Simplex Antisera with Increased Type Specificity in Guinea Pigs by Antibody Suppression (38439)

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(Introduced by H. Ericsson)

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Specific antisera against Herpes simplex virus (HSV) type 1 and type 2 have been produced by cross absorption of rabbit immune sera with heterologous virus antigen (1–4). This method is hampered by the fact that large amounts of virus antigen are necessary and often a nonspecific adsorption of heterologous antibodies may occur. To circumvent these difficulties an alternative method for producing type specific antibodies was applied. The use of passively administered antibody to inhibit an antibody response has been reported previously (for a review see Ref. 5). Using this technique efforts were made to produce type specific immune sera in HSV type 1 and type 2 infected guinea pigs.

Materials and Methods. Tissue culture. BHK 21 clone 13 (6) was kindly supplied by Flow Laboratories Ltd., Scotland, and was grown in 1 liter roller bottles. Eagle's minimum essential medium (MEM), Glasgow modification with 10% tryptose phosphate broth, 7% nonheated calf serum and 2% 0.1 *M l*-glutamine was employed for outgrowth and without serum for maintenance. GMK-AH-1 cells were grown as previously described (2). Guinea pig embryo cell cultures were produced as follows: Guinea pig embryos 3–6 cm in length were minced and trypsinized. The cells were seeded into Roux bottles with the growth medium comprising Eagle's MEM with 10% heated (56°, 60 min) guinea pig serum and 2% 0.1 *M l*-glutamine. For maintenance the same medium without serum was used. All tissue culture media were supplemented with 100 units of penicillin and 100 μg of streptomycin/ml.

Virus strains. HSV type 1, Herpes simplex virus Z was originally obtained from D. C. Gajdusek, National Institute of Neurological Disease, Bethesda, MD and HSV type 2 from G. Plummer, Loyola University, Chicago, IL. The passage history and origin of these strains have been described previously (2).

Preparation of virus stocks. HSV type 1 and type 2 were grown in guinea pig embryo cell cultures for five passages. Virus stocks to be used for inoculation of guinea pigs were prepared as follows. When a complete cytopathic effect had developed the material was frozen and thawed once. The infected tissue culture fluid was centrifuged at 1500*g* for 15 min. Finally 10% dimethylsulfoxide (7) was added to the supernatant fluid and the virus material was kept frozen at −65°. The titer values were $10^{6.0}$ $TCID_{50}$/0.1 ml for HSV type 1 and $10^{5.0}$ $TCID_{50}$/0.1 ml for HSV type 2. For use in plaque neutralization tests HSV type 1 and type 2 were grown in GMK-AH-1 cells. Two days after infection the cultures were harvested as described above. The titer values were 5.6×10^6 plaque-forming units (PFU)/0.1 ml for HSV type 1 and 1.5×10^6 PFU/0.1 ml for HSV type 2.

Plaque titration of virus. Titration of infectious virus and virus-antiserum mixture were performed by a plaque count method with immune serum overlay. The procedure was similar to that of Wheeler *et al.* (8, 9), but tissue culture tubes with GMK-AH-1 cells and overlay with 0.2% pooled human gamma-globulin (10), (Gammaglobulin, Kabi, Stockholm) were used. Each tissue culture tube (11 × 1.6 cm) of GMK-AH-1 cells was exposed for 2 hr at 37° to 0.20 ml of virus diluted in phosphate buffered saline (PBS), pH 7.2 containing 5% heat inactivated fetal bovine serum. The virus inoculum was aspirated and replaced with Eagle's MEM containing 2% heat inactivated fetal bovine serum and 0.2% pooled human gamma-

globulin. After 2 days incubation at 35° the overlay was aspirated and the cells were fixed and stained (8). Plaques were counted with the aid of a stereomicroscope.

Plaque neutralization test. In the neutralization test a constant dose of virus and a varying serum dose were used. It has been shown that this test measures the same antibody activity as in the neutralization kinetics test (11) and that antibodies to the two herpes viruses in the same serum can be quantitated by this technique (11).

Type 1 and type 2 Herpes virus stocks were diluted to give 3000 PFU/ml, and 0.50 ml of HSV was mixed with 0.50 ml of diluted antiserum. Antisera were inactivated for 30 min at 56° prior to use in the neutralization test. After incubation for 1 hr in a water bath at 37° the virus-serum mixture was diluted one in three in cold PBS (0°) and assayed for surviving virus on GMK-AH-1 cells. For each virus-serum mixture three tissue culture tubes were inoculated. Controls consisting of the virus mixture in PBS were included in all tests. In the neutralization test the plot of log percent surviving virus against the quantity of antiserum used yielded a straight line. The least square method was used to fit the best straight line through the experimental points. The degree of neutralization was expressed as a K value calculated from the following formula: $\log V/Vo = -0.43\,Kc$, where V/Vo is the proportion of virus surviving neutralization and c is the antibody concentration expressed as the amount of original serum per unit volume of reaction mixture (12). The relative potency of each serum is indicated by the numerical value of the slope of the straight line. The relative specificity of each serum, however, is best expressed as the ratio of the slopes of lines (13).

Antigens. For use in immunoelectroosmophoresis (IEOP) and complement fixation (CF), viruses were grown in roller bottle cultures of BHK 21 cells as previously described (2). Antigens for IEOP were made by suspending 0.2 ml of packed cells in 1.8 ml of 0.075 *M* barbital buffer, pH 8.6 with 1% Nonidet P 40. This antigen was frozen and thawed three times, centrifuged at 1500*g* for 15 min and the supernatant kept at −25°. Antigens for CF were made by suspending 2 ml of packed cells in 15 ml of barbital buffer (14). This antigen was frozen and thawed three times, heat inactivated at 56° for 20 min, centrifuged at 1500*g* for 15 min and the supernatant kept at −65°.

For immunization of guinea pigs HSV type 1 and type 2 at the fifth passage in guinea pig cells were grown in Roux bottles with embryonal guinea pig cell cultures. When a complete cytopathic effect had developed the infected cells were shaken off the glass and separated from the medium by centrifugation at 1500*g* for 15 min. The packed cells from two Roux bottles were suspended in 3 ml serum free medium and stored at −65°.

Production of hyperimmune serum. Ten guinea pigs (Group A) of both sexes weighing 300–500 g were immunized by infection of the shaved and scarified skin bilaterally on the trunk with HSV type 1 or type 2 virus suspension. HSV type 1 and type 2 were grown in guinea pig cells as outlined above. About two months after the primary infection sc booster injections of HSV antigen grown in guinea pig cells were given to the animals. One week after the booster injection the animals were bled from the ear, and thereafter once or twice each week (15). On each occasion 6–7 ml blood was collected. Sera from animals with high titers in the CF test were pooled. These pools of serum were used for antibody suppression.

Production of type specific antiserum by antibody suppression. Twenty guinea pigs (Group B) of both sexes weighing 300–500 g were divided into four groups and treated as follows. Groups 1 and 2 were infected with HSV type 1 as outlined above. Groups 3 and 4 were infected with HSV type 2. Hyperimmune serum against the heterologous virus type was given intramuscularly in a dose of 1 ml to animals in groups 1 and 3 on the first and second days after infection. The animals were bled 5, 12, 26, 35 and 42 days after infection.

Serological tests. CF tests were performed according to Svedmyr *et al.* (14) IEOP was performed as previously described (2).

Results. Guinea pigs in group A were hyperimmunized with HSV type 1 or type 2. Sera from animals inoculated with the same virus type were pooled. The anti HSV type 1 pool was tested in CF and had a titer of 512 against HSV type 1 and type 2 antigen. The anti-HSV type 2 pool had a titer of 128 against HSV type 1 and type 2 antigen in CF.

The results obtained in CF tests of sera from

animals in group B are summarized in Table I. For HSV type 1, nine out of 14 sera from antibody suppressed animals showing any titer to the homologous antigen, and three out of 17 sera from controls showing any titer to the homologous antigen showed no reaction to heterologous antigen in CF. The sera represent bleedings at different times from the individual animals within each group. The difference is almost significant ($P < 0.05$) (16). For HSV type 2, seven out of seven sera from antibody suppressed animals and three out of 17 sera from controls showed no reaction to heterologous virus antigen in CF. The difference is highly significant ($P < 0.001$). Sera showing type specificity from antibody-suppressed and control animals were selected and pooled. These pools were tested in checker-board titrations, the results of which are shown in Table II. In CF the antibody-suppressed serum pools gave no reaction for anti-HSV type 1 serum and only minor reactions for anti-HSV type 2 serum with heterologous antigen. When tested in CF control serum pools reacted both with HSV type 1 and type 2 antigens although the titers against heterologous antigen were relatively low.

These four serum pools were also tested in IEOP (Table III) and plaque neutralization tests (Fig. 1). Before use in IEOP, aliquots of the serum pools were concentrated four times with "Lyphogel" (Gelman Instruments, Ann Arbor, MI). When tested prior to concentration the antibody-suppressed serum pools only reacted with homologous antigen, the anti-HSV type 1 serum giving one and the anti-HSV type 2 serum, two precipitin lines with homologous antigens. The concentrated anti-HSV type 2 serum cross reacted with heterologous antigen, whereas concentrated anti-HSV type 1 serum did not. Serum pools from control animals reacted to the same extent with both the homologous and heterologous antigens.

In plaque neutralization tests the antibody-suppressed serum pools did not show increased specificity in comparison with serum pools from control animals (Fig. 1). The relative specificity of each serum pool can be expressed as the ratio of the slopes of lines. The ratio between K values for homologous and heterologous virus for anti-HSV type 1 were 15.6 and 14.9 for serum pools from antibody suppressed and control animals respectively. The corresponding figures for anti-HSV type 2 were 7.1 and 5.6.

Discussion. Preliminary attempts to produce type specific antisera in rabbits by antibody suppression were unsuccessful. This is in agreement with findings of Uhr and Baumann (17). However, in guinea pigs it was possible by means of antibody suppression to produce antisera against HSV type 1 and type 2 of enhanced specificity as measured in CF and IEOP, although in plaque neutralization tests no significant difference in specificity could be demonstrated between antisera made with and without antibody suppression. The guinea pig antisera were highly specific in neutralization tests even without antibody suppression and any modification induced by passive administration can only be of minor order. These antisera can be used in IEOP (2) without absorption procedures for the typing of HSV strains. The precipitin patterns observed in IEOP indicate the presence of at least one HSV type 1 and two HSV type 2 specific antigens. Using absorbed antisera other investigators (1, 2, 18) have found that HSV type 1 has two to six and HSV type 2 one specific antigen. These discrepancies might be explained by the use of antisera produced by different techniques and in different animals. In particular, the anti-HSV type 1 serum produced by antibody suppression showed a high degree of specificity and potency while the specificity of anti-HSV type 2 serum was increased but to a lower degree than for anti-HSV type 1 serum.

It is well known that passive administration of antibodies is an effective means for suppression of antibody production against a variety of antigens and antigen mixtures (5). The effect of the immune response to active bacteria or virus has been less extensively studied. Corbel (19) has described passive administration to guinea pigs of homologous antiserum of high titer to *Brucella abortus* prior to vaccination with *B. abortus*. Complete suppression of the primary serological response was reported as measured by CF and other serological tests. In the present study the effect of antibody suppression in guinea pigs infected with HSV type 1 and type 2 was incomplete.

After HSV type 1 infection and passive administration of heterologous antibody HSV type 1 antibodies of high specificity were produced in two animals. After HSV type 2 infection and passive administration of heterologous antibody, two out of four animals produced moderately specific HSV type 2 antibodies. The anti-

TABLE I. Effect of Passively Administered Heterologous Antibody on the Immune Response to Herpes Simplex Virus Type 1 and Type 2 in the Guinea Pig.

			Titers in complement fixation against									
			HSV type 1 antigen					HSV type 2 antigen				
	Animal no.	Immunization schedule	Number of days after virus infection					Number of days after virus infection				
			5	12	26	35	42	5	12	26	35	42
Group I	1	HSV type 1	<4	<4	4	8	8	<4	<4	8	4	<4
	2[a]	infection + 2 ml	<4					<4				
	3	of anti-HSV	<4	32	64	8	—	<4	<4	32	<4	—
	4	type 2 hyperimmune	<4	16	128	32	4	<4	<4	8	8	<4
	5	serum im	<4	8	32	32	64	<4	<4	<4	<4	<4
Group II	6	HSV type 1	<4	128	128	128	—	<4	32	128	32	
	7	infection	<4		≥256	64	64	<4		32	16	4
	8		<4	16	64	64	32	<4	<4	16	4	4
	9		<4	16	128	64	—	<4	32	128	32	—
	10		<4	32	128	16	4	<4	<4	64	4	<4
Group III	11	HSV type 2	<4	<4	<4	<4	<4	<4	<4	32	16	16
	12[a]	infection + 2 ml	<4					<4				
	13	of anti-HSV	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4
	14	type 1 hyperimmune	<4	<4	<4	<4	<4	<4	8	16	16	16
	15	serum im	<4	<4	<4	<4	—	<4	<4	<4	<4	—
Group IV	16	HSV type 2	<4	<4	<4	4	—	<4	<4	8	4	—
	17	infection	<4	<4	32	16	—	<4	32	64	4	—
	18		<4	64	16	4	4	<4	64	8	8	4
	19		<4	32	≥256	8	8	<4	32	128	16	16
	20		<4	<4	≥256	16	8	<4	4	64	16	16

[a] Died between 5 and 12 days after infection. Symbol: —, not tested.

TABLE II. Checker-Board Titration in Complement Fixation Test of Serum Pools from Animals to which Heterologous Antibody was Administered and from Controls.

		Pools of antibody suppressed sera							Pools of sera from control animals						
		HSV type 1 serum pool antiserum dilutions[a]							HSV type 1 serum pool antiserum dilutions						
	Antigen dilutions	4	8	16	32	64	128	256	4	8	16	32	64	128	256
HSV type 1 CF antigen	1/2	+	+	+	+	±	−	−	+	+	+	+	+	−	−
	1/4	+	+	+	+	±	−	−	+	+	+	+	+	−	−
	1/8	+	+	+	+	±	−	−	+	±	−	−	−	−	−
	1/16	−	−	−	−	−	−	−	+	−	−	−	−	−	−
HSV type 2 CF antigen	1/2	−	−	−	−	−	−	−	+	+	±	−	−	−	−
	1/4	−	−	−	−	−	−	−	+	±	−	−	−	−	−
	1/8	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1/16	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Uninfected control-antigen	1/2	−	−	−					−	−	−				
		HSV type 2 serum pool antiserum dilutions							HSV type 2 serum pool antiserum dilutions						
	Antigen dilutions	4	8	16	32	64	128	256	4	8	16	32	64	128	256
HSV type 1 CF antigen	1/2	+	±	−	−	−	−	−	+	+	±	−	−	−	−
	1/4	±	−	−	−	−	−	−	+	+	±	−	−	−	−
	1/8	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1/16	−	−	−	−	−	−	−	−	−	−	−	−	−	−
HSV type 2 CF antigen	1/2	+	+	+	±	−	−	−	+	+	+	±	−	−	−
	1/4	+	+	+	−	−	−	−	+	+	+	±	−	−	−
	1/8	+	−	−	−	−	−	−	+	±	±	−	−	−	−
	1/16	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Uninfected control-antigen	1/2	−	−	−					−	−	−				

[a] Dilutions are expressed as reciprocals.

TABLE III. Titrations of Antigen and Antiserum in Immunoelectroosmophoresis.

Antigen dilution	Antibody suppressed serum pools[a]			
	HSV type 1 serum		HSV type 2 serum	
	4 times concentrated	unconcentrated	4 times concentrated	unconcentrated
BHK 21 uninfected control antigen				
1/1	0	0	0	0
1/4	0	0	0	0
1/16	0	0	0	0
HSV type 1 antigen				
1/1	++	+	++	0
1/4	+	(+)	+	0
1/16	0	0	0	0
HSV type 2 antigen				
1/1	0	0	++	+
1/4	0	0	0	(+)
1/16	0	0	0	0

[a] 0, no precipitate; (+), very faint precipitate visible only by fluorescence (2); +, very faint precipitate; ++, distinct precipitate. Precipitin reactions read after staining.

body production of all animals was followed for several months. With increasing time the specificity of the antibodies diminished. Five to 6 mo after infection no animal produced antibody with increased specificity as compared with controls. A changing pattern of antibody production with time from specific to nonspecific might be explained by a continuous or intermittent antigenic stimulation of the immunological system. Such an antigenic stimulation might be caused by the well known persistence of HSV after infection. It is known that priming or sensitization of the immune system is more difficult to inhibit by passive antibody administration than the primary antibody response (5). By immunizing guinea pigs with noninfective HSV antigen and passive administration of heterologous antibody the difficulties outlined above might be avoided and type specific antisera of more complete specificity and higher potency could possibly be produced.

An increase of antibody affinity and a decrease of specificity is well known to occur during the immune response. This might be an alternative explanation to the appearance of heterologous antibody.

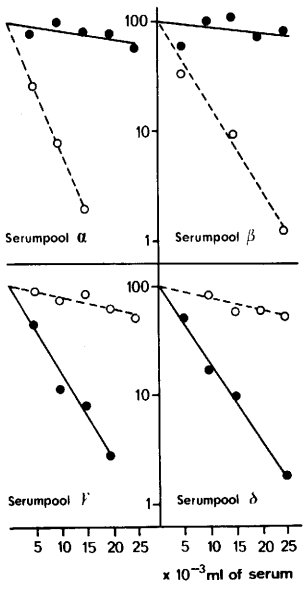

FIG. 1. Multiplicity analysis of neutralizing potency of anti-HSV type 1 and type 2 serum pools from animals to which heterologous antibody was administered and from controls. The neutralization tests were performed as described under "Materials and Methods." Each point is an average for triplicate tissue culture tubes. The same virus preparations were used in all tests. ---------, neutralization of HSV type1; ————————. neutralization of HSV type 2. Serum pool α: anti-HSV type 1 serum pool from antibody suppressed animals. Serum pool β: anti-HSV type 1 control serum pool. Serum pool γ: anti-HSV type 2 serum pool from antibody suppressed animals. Serum pool δ: anti-HSV type 2 control serum pool.

Summary. Passive administration of heterologous antibody to guinea pigs infected with Herpes simplex virus type 1 and type 2 allowed a production of antisera of enhanced specificity. The anti Herpes simplex virus type 1 serum produced was of high specificity and potency and showed only minor reactions with Herpes simplex virus type 2. The anti-Herpes simplex virus type 2 serum produced was of moderate specificity, but clearly differentiated between Herpes simplex virus type 1 and type 2 in different immunological reactions. The procedure described is a simple method whereby relatively specific antisera for typing of Herpes simplex virus type 1 and type 2 strains can be produced.

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