Acta virol. 34 : 305—320, 1990

SPREAD OF HERPES SIMPLEX VIRUS (HSV) STRAINS SC16, ANG, ANGpath AND ITS glyC MINUS AND GlyE MINUS MUTANTS IN DBA-2 MICE

J. RAJČÁNI, U. HERGET*, H. C. KAERNER*

*Deutsches Krebsforschungszentrum, Institut für Virusforschung, 6900 Heidelberg, F.R.G., Institute of Virology, Slovak Academy of Sciences, 84246 Bratislava, Czechoslovakia

Received August 17, 1989

Summary. — Herpes simplex virus type 1 (HSV-1) strains SC16, ANG, its pathogenic variant ANGpath and the mutants ANG--pathgC18 glycoprotein C (glyC) negative and ANGpathI2-4 (glyE negative) were compared for their ability to spread in DBA-2 mice after peripheral inoculation. Virus infectivity assay in 9 organs at days 2, 3, 4, 5, 6, and 10 post-infection (p.i.) and morphologic examinations (immunofluorescence, PAP staining) showed the following: SC16, ANG, and ANGpath spread first (days 2—3 p.i.) by haematogenic route to spleen, liver, and adrenal gland. Since day 4 the invasion of the vegetative and peripheral nervous system took place in SC16 and ANGpath-infected mice, followed by virus spread to the spinal cord and brain stem. In ANG-infected mice the invasion of peripheral nervous system was minimal although both ANG as well as ANGpath spread along the axons. In ANG pathgC18-infected mice a relatively prolonged viraemic phase (days 2—4 p.i.) represented with foci of virus antigen-containing cells in spleen, liver, and mesenterial connective tissue was accompanied with a low grade invasion of the peripheral nervous system (days 3—4 p.i.). No spread by any route of ANGpathI2-4 was observed after intraperitoneal inoculation. When comparing ANGpath and SC16, the latter seemed slightly more lethal, since ANGpath killed 67.2% of DBA-2 mice which were given 2×10^6 PFU/0.1 ml by i.p. route as compared to the 100% lethality of SC16-infected animals.

Key words: *herpes simplex virus*; *pathogenesis*; *mutants*; *neural spread in mice*

Introduction

Two decades elapsed since the pilot studies of the neural spread of HSV in mice as revealed by immunofluorescence (Yamamoto *et al.*, 1965; Rajčáni *et al.*, 1969) and electron microscopy (Yamamoto *et al.*, 1973). While in suckling mice the haematogenous dissemination of the virus was striking

(Johnson, 1964), the neural spread prevailed in mature animals (Johnson and Mims, 1968; Rajčáni *et al.*, 1970). In the susceptible host, Schwann cells of peripheral nerves replicated the virus (Rabin *et al.*, 1968; Severin and White, 1969; Rajčáni and Conen, 1972; Lascano *et al.*, 1982), while the axonal spread was more evident only under restrictive conditions in less susceptible animals (Baringer and Griffith, 1970; Kristensson *et al.*, 1971; Hill *et al.*, 1972; Cook and Stevens, 1973).

It has been shown that the outcome of HSV-infection depends both on the properties of the host and on the virus strain used (Lopez, 1975; Kirchner *et al.*, 1978). We decided to repeat the classical experiments using virus strains of divergent pathogenicity and neurovirulence. Strain ANG is non-pathogenic for DBA mice by i.p. inoculation. The peripherally pathogenic ANGpath variant prepared by 12—15 intracerebral passages (Kaerner *et al.*, 1983; Kümel *et al.*, 1986) was found distinct from ANG by following features: 1. ANG did not reach the spinal cord and brain stem of DBA mice if given in a high dose of 2×10^6 PFU although ANGpath spread there when given in a 10 times lower dose of 2×10^5 PFU. 2. At early stages p.i. both variants multiplied in the peritoneal cavity and spread to visceral organs such as spleen ond others (Kümel *et al.*, 1982; Schröder and Kümel, 1986). 3. As previously shown, both ANG and ANGpath spread from peritoneal cavity to spleen and visceral organs (Kümel *et al.*, 1986; Kümel and Schröder, 1986) but the invasion of ANG into the nervous system did not occur although the latter was capable to spread by neural route.

For comparison, we used the SC16 strain which is pathogenic for several strains of mice and is widely used in the ear model of HSV latency (Harbour *et al.*, 1981). Finally two mutants of ANGpath were included into these experiments, namely the glyC minus mutant ANGpathgC18 and the glyE minus mutant ANGpathI2-4, to investigate the possible role of these glycoproteins in virus spread.

Materials and Methods

Virus strains. The SC16 strain (Hill et al., 1975) was kindly provided by T. E. Hill, University of Bristol, U.K.The strains ANG and ANGpath, the pathogenic variant of ANG prepared by serial intracerebral passages of ANG, were characterized previously (Kaerner *et al.*, 1983). The glyC negative mutant ANGpathgC18 (Weise, 1987) did not express glyC, the preparation of the deletion mutant ANGpathI2-4 has been described elsewhere (Neidhardt *et al.*, 1987). All viruses were grown in Rita cells and stored at −70 °C until use.

Animals. DBA-2 mice 4—6 weeks old (Deutsche Versuchstieranstalt, Hannover) were kept in an isolator on a standard diet. The animals were infected by i.p. route with 0.1 ml virus suspension as follows: 2×10^5 PFU of SC16 (20 mice), 2×10^6 PFU of ANGpath (35 mice), 2×10^7 of ANG (25 mice), 2×10^6 PFU of ANGpathgC18 (20 mice), 2×10^6 PFU of ANGpathI2-4 (20 mice). On days 2, 3, 4, 5, 6, and 10 p.i., 2—3 mice were sacrificed for virus assay and morphological examinations.

The quick axonal transport was tested by inoculation of DBA-2 mice into the right lip and cornea with ANGpath (30 mice, 1×10^7 PFU in 20 µl, respectively), ANG (10 mice, 1×10^7/20 µl), ANGpathgC18 (10 mice, 2×10^6 PFU/20 µl) and ANGpathI2-4 (10 mice, 1×10^7 PFU/20 µl). By 22 hr after virus administration both trigeminal ganglia were removed and cultured for 10 days.

Virus assay. Suspensions were made from liver (2 ml), kidneys (1 ml), adrenal glands (0.5 ml),

spinal cord (lumbal segments, 0.5 ml), spleen (1 ml), brain stem (0.5 ml), brain (1 ml), lungs (1 ml), and trigeminal ganglia (0.5 ml) of i.p. infected mice. The organs were washed in sterile distilled water, homogenized, sonicated (3 times 20 sec), centrifuged (10 min, 3500 rev/min) and tested for virus presence in BHK cells grown on 24-well plastic microplates in medium BME supplemented with 8% calf serum and antibiotics. Results were read 2 and 3 days after incubation at 37 °C in 5% CO_2 atmosphere. Microfoci of CPE were counted at the limiting dilution and the results were expressed in $TCID_{50}$ units per organ.

The medium fluid from the explanted ganglion samples was exchanged on days 3 and 7 in culture. The explantation was finished on day 10 in culture. The medium samples and the homogenized fragments collected on day 10 in culture were tested for infectious virus in BHK cells.

Explantation procedure. By 22 hr p.i. both trigeminal ganglia were removed, minced, and cultured in plastic Petri dishes in medium RPMI-1640 supplemented with 10% foetal calf serum and antibiotics.

Immunofluorescence (IF). Blocks were quickly frozen in liquid nitrogen from following organs and areas of mouse body: liver, spleen, kidneys, and adrenal gland (including the retroperitoneal vegetative nerves and ganglia), lungs, and heart (including mediastinal nerves and ganglia), lumbal segments of the spine including spinal cord, spinal routes and ganglia, and the sympathetic paravertebral ganglia, brain stem and cerebellum, the rest of brain, both trigeminal ganglia. Cryostat sections (6-7 μm thick) were cut from each block at three levels about 300 μm apart. From each level at least 6 sections were sticked to 2 slides, air dried, fixed in acetone, and stained with rabbit immune serum to HSV-1 diluted 1 : 100 (Ortho-Diagnostic) and an anti-rabbit conjugate (goat, anti-rabbit, IgG fraction, FITC labelled, Dianova) diluted 1 : 50. Before staining with immune serum the sections were treated with normal goat serum diluted 1 : 20. Control slides were not stained with anti-HSV immune serum. Finally, the sections were counterstained with 0.1% thiasin red solution (Fluka) for 2 min and mouted into Elvanol. The preparations were viewed in a Zeiss microscope.

PAP staining. Organs and tissues as listed above were fixed in Carnoy solution or alternatively in neutral buffered formalin (4%) and embedded into paraffin. Blocks were cut at room temperature, deparaffinized (xylol, alcohol) treated with methanol/hydroperoxide (3% H_2O_2) to remove the endogenous peroxidase for 20 min, and with normal swine serum (Ortho) diluted 1 : 10 for 20 min to block non-specific staining. Then the sections were stained with the rabbit immune anti-HSV-1 serum (see above), with the bridging swine anti-rabbit IgG, and finally, with the PAP complex (rabbit PAP, Dianova; at a concentration of 50 μg/ml for 45 min). All reagents were applied at room temperature. The reaction was visualized with aminoethylcarbazol (0.4%) solution containing H_2O_2 (0.02%) for 25 min and then the sections were stained with haematoxylin for 30 sec and mounted into glycerine-gelatine (Merck). Control sections were stained omitting the immune rabbit anti-HSV-1 serum. To improve the staining of the HSV antigens parallel sections from the formalin embedded material were stained also after digestion with pronase (1 mg/ml for 10 min, room temperature).

Results

Spread of infectious virus

The SC16 strain given in the dose of 2×10^5 PFU per mouse by i.p. route was lethal in each case. Between days 2—4 p.i., the presence of virus in spleen, liver, and lungs was typical for the viraemic phase, while during the second phase (days 4—7 p.i.) invasion of the CNS became apparent (Table 1). The adrenal gland contained virus in extremely high titres throughout.

Table 2 shows the distribution of ANGpath and ANG in the same organs of DBA-2 mice. Although ANG was administered in a 10-times higher dose than ANGpath (lethality 67.2%), none of ANG-infected mice died. Viraemic phase with involvement of kidney, liver, and lungs was present in both ANG as well as ANGpath-infected mice, but only in the latter has the virus

Table 1. Distribution of HSV in the organs of DBA-2 mice after i.p. inoculation of 2×10^5 PFU strain SC16

Organ	Days post-infection 2	4	7
Spleen	1×10^2	1×10	
Liver	6×10^3	1×10^2	
Lungs	1×10^4	1×10	
Kidney	6×10^3	6×10	
Adrenal gland	5×10^5	5×10^6	5×10^2
Spinal cord	5×10^3	3×10^3	6×10^3
Brain stem		5×10^2	3×10^3
Brain			
Trigeminal ganglion		2×10^2	6×10^0

Lethality	Days post-infection 2	3	4	6	7	8	Total
				4	3	1	8/8

reached the spinal cord and brain stem. The adrenal gland was positive throughout, but not in ANG-infected mice, which did not show any virus in the organs tested on days 6 — 10 p.i.

None of the mutants-infected mice died. Virus assays were performed on days 2, 4, 6, and 10 p.i.; ANGpathgC18 was found in small amounts in the spleen and traces of this virus were occasionally detected in the trigeminal ganglion.

No virus spread was noticed in mice infected with ANGpathI2-4.

Distribution of SC16 and ANGpath antigens in i.p. infected mice

Several mononuclear cells showed positive IF on day 2 p.i. in the peritoneal cavity. In the mesenterial and retroperitoneal connective tissue and fat tissue fluorescence was seen in the fibrocytes and adipose cells. Scattered groups of hepatocytes contained the antigen in the liver (Fig. 1) as confirmed by PAP staining. The foci were located either in the lobules, or in the vicinity of venules, or near to the portobiliar spaces. Occasional necrosis of the hepatocytes was accompanied with discrete mononuclear infiltrates. In the spleen HSV antigen was detected mainly in the red pulp (mononuclear cells, reticulum cells) while the follicles (B lymphocytes) were predominantly negative. No virus antigen was found in the lungs and kidneys. The gut wall showed numerous positive fibrocytes in the subserosa, and positive smooth muscle cells in the outer longitudinal layer. The latter feature was more prominent on day 4 p.i. and was also confirmed by PAP staining. Positive IF and PAP staning of the HSV antigen was found in the adrenal gland cortex

Table 2. Distribution of ANGpath and ANG strains after i.p. inoculation of DBA-2 mice

Organ	ANGpath					ANG			
	day 2	day 3	day 4	day 6*	day 6	day 2	day 4	day 6	day 10
Spleen	7×10^3	1×10^2	3×10^3						
Liver	1.5×10^2	3×10^3							
Lungs	7×10	6×10	3×10^2	3×10^3		1×10^2			
Kidney	7×10^3	1×10^2				5×10			
Adrenal gland					3×10^2	3×10^3			
Spinal cord		2×10^5	5×10^5	3×10^4	3×10^3	1×10^6	2×10^5		
Brain stem			2×10^4	2×10^2	2×10^3				
Brain (other areas)			3×10^4	2×10^4	2×10^2				
Trigeminal ganglion									

	Days post-infection							Days post-infection					
Lethality	2	3	4	5	6	7	Total	2	3	4	5	7	Total
			3	2	4		10/14*						0/16

* additional moribund animal included into the total lethality

and medulla throughout. On day 7 the heavy involvement of all three cortical layers and parenchymal cells of the medullar trabecules was prominent (Figs. 3, 5). In addition, the antigen was present in the endothelium cells of medullar sinuses. At higher magnification, vacuolization and necrosis of parenchymal cells in the cortex was evident, the antigen was present in nuclei and cytoplasm of parenchymal cells which showed less extensive destruction and typical nuclear inclusion bodies.

Since day 4 p.i. the involvement of the mesenterial vegetative nerves and ganglia became striking. The Schwann cells of retroperitoneal nerves showed positive fluorescence as well as nerves of lumbar plexus, the spinal roots, not excluding such distant vegetative nerves as n. vagus in its mediastinal coarse. The sympathetic paravertebral ganglia as well as the large coeliac and other paraaortal ganglia contained neurons filled with antigen (Figs. 2, 4). In addition to the vegetative ganglia, spinal ganglia and spinal cord were also involved. Spinal cord was positive mainly in SC16-infected mice (Table 3). On day 7 viral antigen was seen in motoneurons of the anterior horns as well as in neurons of the lateral horns at the level of lumbar segments

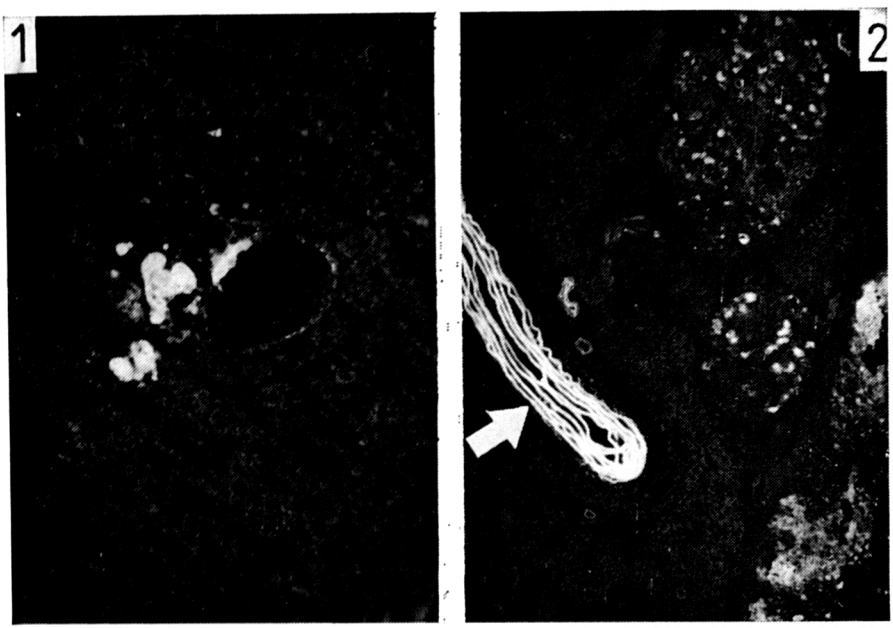

Fig. 1. ANGpath strain, day 2 p.i. Blood borne focus showing positive immunofluorescence of HSV antigens in the hepatocytes near to a venule and in its endothelium cells. Indirect IF, magn. × 300.

Fig. 2. ANGpath strain, day 6 p.i. HSV antigens in the adrenal cortex in the Schwann cells and neurons of retroperitoneal vegetative nerves and ganglia. Arrow indicates the elastica or a large artery, conterstained with thiasin red. Indirect IF, magn. × 220.

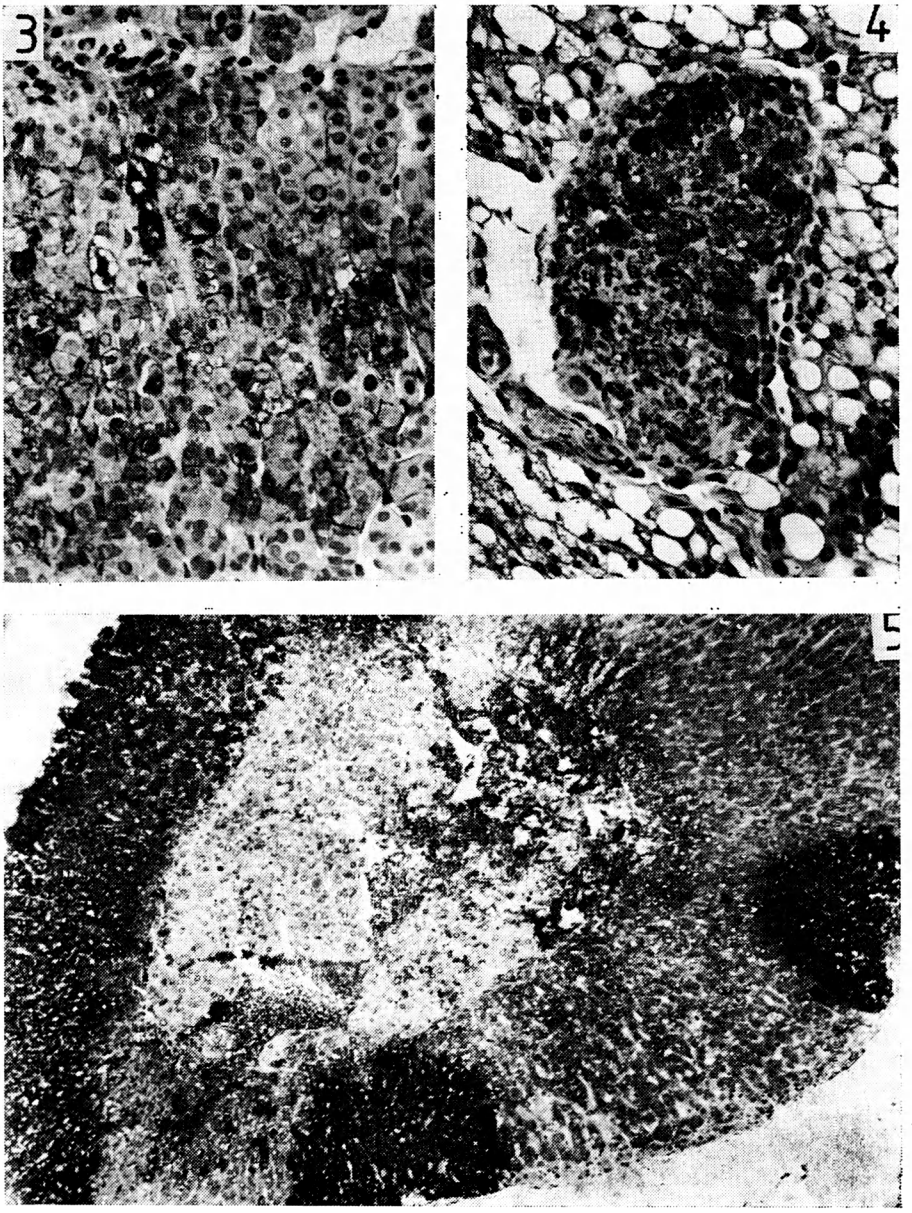

Fig. 3. Strain ANGpath, day 4 p.i. Necrosis and vacuolization of parenchymal cells in the zona fasciculata and reticularis of adrenal gland cortex; several cells contain nuclear inclusions. HSV antigens (dark granules) in the cytoplasm and nuclei. PAP stain, magn. × 300.

Fig. 4. ANGpath, day 6 p.i. Vegetative retroperiteneal ganglion with neurons containing HSV

Table 3. Distrubition of SC16 virus antigen in DBA-2 mice as determined by immunofluoreseence and PAP staining

	Days post-infection					
	2 (IF)	2 (PAP)	4 (IF)*	4 (PAP)*	7 (IF)	7 (PAP)
Mesenterium (connective tissue)	+					
Gut wall (smooth muscle, intramural nerves)	+		+	+		
Spleen	+					
Liver	+	+				
Adrenal gland	+	+	+	+	+	+
Retroperitoneal vegetative nerves			+	+	+	+
Retroperitoneal vegetative ganglia			+	+	+	+
Spinal ganglia and nerves (lubar segments)			+		+	+
Spinal cord					+	+
Mediastinal vegetative nerves (vagus)					+	
Brain stem				±**	+	+
Brain: thalamus						+
hypothalamus						+
basal ganglia						+
cortex						+

* in 2 mice ** a few positive glial cells

and in glial cells of the white and gray matter. The involvement of brain stem was more prominent with the SC16 strain. HSV antigen was found in neurons of reticular formation, *nc. abiguus*, vestibular nuclei and *nc. terminalis n. trigemini* and many other unidentified structures. The higher brain structures were affected in mice infected with the SC16 strain only (Table 3). Thus, the thalamic nuclei, hypothalamus, and brain cortex showed positive neurons in the SC16-infected animals but not in the ANGpath infected ones (Table 4).

Distribution of virus antigen in ANG-infected DBA mice

The findings are summarized in Table 5. The presence of the antigen in mononuclear cells of the peritoneal cavity, in mesenterial and in retroperitoneal connective and adipose tissues was similar as in the previous animal groups. The HSV antigen containing cells were seen in the spleen (reticulum cells), liver (hepatocytes), intestinal wall (smooth muscles), and adrenal gland (parenchymal cells) on day 2 p.i. On day 4 minimal fluorescence was found in a few Schwann cells of a mesenterial nerve, the vegetative ganglia, the nerves of lumbar plexus, the spinal cord, and brain stem were negative.

antigens. Note necrosis of neurons and mononuclear infiltration within ganglion as well as in surrounding fat tissue. PAP method, magn. × 300.

Fig. 5. SC16 strain, day 7 p.i. Extensive staining of HSV antigens in the adrenal cortex and medulla. PAP method, magn. × 120.

Table 4. Detection of ANGpath antigens in intraperitoneally infected DBA-2 mice by IF and PAP staining

Organ (tissue)	Days post-infection								
	2 (IF)	2 (PAP)	3 (PAP)	4 (IF)	5 (PAP)	6 (IF)	6 (IF)*	6 (PAP)	10 (IF, PAP)**
Messenterial connective tissue	+		±						
Spleen	+								
Liver	+	+	±	±					
Adrenal gland	+	+	+	+	+	+	+	+	
Retroperitoneal vegetative nerves and ganglia				+	+		+	+	
Spinal ganglia and roots lumbal segments							+		
Intestinal wall (smooth muscles)	+		+	+					
Mediastinal nerves (vagus)							+		
Lungs							+		
Brain stem							+		
Brain (other areas)									

* Dead mouse ** 2 mice

Table 5. Detection of HSV antigens in ANG-infected DBA-Z mice by IF and PAP staining

Organ	Days post-infection				
	2 d. (IF, PAP)	4 d. (IF, PAP)	5 d. (IF, PAP)	6 d. (IF, PAP)	10 d. (IF, PAP)
Mesenterial connective tissue	±				
Spleen	+				
Liver	+	±			
Adrenal gland	+	+			
Retroperitoneal vegetative nerves and ganglia		±			
Spinal roots and ganglia					
Mediastinal veg. nerves					
Spinal cord					
Brain stem					
Brain (other areas)					
Lung					
Intestinal wall	+	±			

No virus antigen was found at later intervals p.i. The morphological and virological findings in ANG- and ANGpath-infected mice are compared in Table 7.

Virus-specific antigens in DBA-2 mice infected with different ANGpath mutants

Table 6 shows that HSV antigen was present in the spleen (reticulum and mononuclear cells; compare above) of the animals which had been infected with the both mutants. Minimal positivity was seen in mice infected with the ANGpathgC18 mutant in the mesenterial and retroperitoneal connective tissues, in vegetative nerves and in adrenal gland on days 5—6 p.i.

Acute axonal spread of ANG, ANGpath, and of its mutants

To test the axonal transport from input virus the mice were inoculated with a high virus dose into the right scarified cornea and lip (a total volume of 20 μl). By 22 hr later, both trigeminal ganglia were cultured to prove whether the virus which had reached the ganglion but had not started replication in its neurons would then multiply in the cultured ganglion explants. When the right trigeminal ganglia of 10 mice inoculated with 1×10^7 PFU of ANGpath were cut in semiserial sections and examined for the presence of HSV antigen, this was found in a single pseudounipolar neuron in one mouse only; the axonal transport occurred at least in 7 mice (Table 8). Alternatively, the quick axonal transport took place in 6 ANG-infected mice, but in none of those inoculated with the glyE negative deletion mutant. Using

Table 6. Detection of HSV-antigens in ANGpathgC18 and ANGpathI2-4 mutants-infected DBA-2 mice by IF and PAP staining

Organ	ANGpathgC18				ANGpathI2−4			
	day 3*	day 5*	day 6+	day 10+	day 3*	day 5*	day 6+	day 10+
Mesenterial connective tissue			+		±			
Intestinal wall								
Spleen	+				+			
Liver		±						
Adrenal gland								
Retroperitoneal vegetative nerves and ganglia		±						
Spinal ganglia and cord								
Brain stem								
Brain(other areas)								
Mesenterial lymph nodes			+					

* by IF and PAP staining + by IF only

Table 7. Comparison of virus distribution in ANGpath and ANG-infected mice with the morphological findings

	Days post-infection					
	day 2		day 4		day 6	
	ANG	ANGpath	ANG	ANGpath	ANG	ANGpath
Mesenterium	+	+				
Spleen	+	+ (+)		(+)		
Liver	+ (+)	+ (+)	±			
Adrenal gland	+ (+)	+ (+)	+ (+)	+ (+)		+ (+)
Retroperitoneal and paravertebral vegetative nerves			±	+		+
Spinal ganglia and nerves (lumbal segments)						+
Outer smooth muscle layer of the gastrointestinal tract	+	+	+	+		
Mediastinal (remote) vegetative nerves						+
Spinal cord				(+)		+ (+)
Brain stem				(+)		+ (+)

± very few positive cells in one out several sections
(+) in brackets: virus infectivity assay
+ no brackets: IF and PAP staining

Table 8. The quick axonal transport in DBA-2 mice

Virus strain (mutant)	Inoculated dose	RTG	LTG	Days in culture
ANGpath	1×10^7	3/10	0/10	4
		7/10	1/10	7
		7/10	1/10	10
ANGpath	8×10^5	0/10	0/10	4
		0/10	0/10	7
		0/10	0/10	10
ANG	1×10^7	0/10	0/10	3
		0/10	0/10	7
		6/10	2/10	10
ANGpathgC18	2×10^6	0/10	0/10	3
		0/10	0/10	7
		0/10	0/10	10
ANGpathI2-4	1×10^7	0/10	0/10	4
		0/10	0/10	7
		0/10	0/10	10

RTG, LTG = right (left) trigeminal ganglion

a lower dose of ANGpath the explantation results were negative probably because there was no neuritic uptake from virus input in scarified cornea due to the prevalence of HSV receptors on corneal epithelium cells. As the stock titre of ANGpathgC18 was lower than that of ANGpath, it was not possible to use this mutant in a higher dose than 2×10^6 PFU. Thus we could not draw a final conclusion whether glyC was involved in effective neuritic uptake and/or axonal transport, which has certainly failed the case of the glyE minus mutant.

Discussion

The results of the present study indicated that upon peripheral (i.p.) infection there was a minimal invasion of the peripheral (vegetative) nervous system with ANG in contrast to ANGpath and SC16-infected mice, in which the invasion of peripheral nerves and ganglia was overwhelming since day 4 p.i. The minimal involvement of Schwann cells of peripheral nerves in the presence of axonal spread (as proved by explantation of the trigeminal ganglion 22 hr after intracorneal and lip inoculations), was not accompanied by transneuronal transfer of ANG to the second neuron in the spinal cord and brain stem. This confirms the notion that only neurovirulent strains progress beyond the ganglion to the CNS, thus a postganglionic block exists for HSV-1 ANG and/or other nonencephalitogenic strains (Kümel *et al.*,

1986). In full agreement with the abovementioned results, we confirmed the quick axonal spread of ANG. However, in our hands, the Schwann cells of peripheral and vegetative nerves, the satellite cells and neurons of vegetative and sensory ganglia showed a restrictive behaviour to ANG but not to ANGpath and SC16. In DBA-2 mice infected by oral and corneal routes ANGpath multiplied in the pseudounipolar neurons of the trigeminal ganglion (manuscript in preparation). Thus, while both ANG and ANGpath spread along axons and established latency in the ganglion cells (Kümel *et al.*, 1986), only ANGpath could overcome the restrictive behaviour of neural cells as evidenced by synthesis of virusspecific antigens in the Schwann cells of peripheral nerves, satellite cells and neurons of vegetative and sensory ganglia and in glial cells and neurons of the CNS. Necrotizing lesions develop in the trigeminal root entry zone after oral/ocular HSV inoculation of neuropathogenic strains (Towsend and Baringer, 1978). In A/J mice infected with the HSV-1 F strain into the tongue, the virus entered the neuromuscular junctions, travelled along the axons to the hypoglossal nucleus in the brain stem and at the peripheral to central nervous system junction it entered the astroglial processes (Openshaw and Ellis, 1983). In Balb/c mice infected with the SC16 strain the transneuronal transfer was combined with a local cell to cell transfer in the brain stem via the glial elements (Ugolini *et al.*, 1987). In our hands using susceptible outbred juvenile mice infected by oral route, the Schwann cells and the oligodendroglial cells contained virus antigen at both sides of the Redlich-Obersteiner junction line marking the transition of the peripheral nervous system to CNS (Szántó and Rajčáni, 1976). In the present study ANGpath and especially SC16 showed not only a simple transneural axonal spread and multiplied actively in the next neuron, but they grew also in the satellite cells of ganglia, in glial cells of CNS and in the Schwann cells of the peripheral nerves. Especially the ability to multiply in the Schwann cells of peripheral nerves and in the ganglion satellites contributed to significant enhancement of virus spread.

The involvement of adrenal gland was repeatedly described in HSV-infected mice (Rajčáni *et al.*, 1970; Nachtigal and Caufield, 1984; Potratz *et al.*, 1986; Irie *et al.*, 1987). The virus enters adrenal medulla and cortex by blood-stream from the sinuses to spread further to the coeliac and other retroperitoneal vegetative ganglia (first neuron) and then to the sympathetic neurons of the lower thoracic cord (second neuron) (Hill *et al.*, 1986). Again, SC16 as well as ANGpath extensively multipled in the adrenal gland and spread to the coeliac and other vegetative ganglia and spinal cord, while ANG following a limited replication in the adrenal gland did not spread along nerves, at least not in a significant amount.

The comparison of the glyC- and glyE-minus mutants with the progression the maternal ANGpath strain showed the following:

1. The glyE minus mutant (ANGpathI2-4) multiplied in a very limited extent in spleen without spreading to other organs. No neuritic uptake and no axonal spread was found when testing the quick axonal transport by explanting the trigeminal ganglion by 22 hr after oral/corneal inoculation.

Restriction of glyE minus HSV mutants was believed to be mediated by immune mechanisms. Because glyE is one essential component of the Fc receptor its expression on the surface of infected cells has been suggested to interfere with the cytotoxic and/or natural killer immune destruction of these cells (Adler *et al.*, 1978; Courtnery *et al.*, 1984). The binding of any immunoglobulin molecules to the surface of infected cells may hamper the access of virusspecific antibodies participating in the antibody and complement mediated cytolysis. In addition, the binding of immunoglobulins to the Fc receptor would reduce the yield of infectious virus produced in the cell (Costa *et al.*, 1977). Thus, it can be assumed that the extremely limited replication of the glyE minus mutant at the portal of entry reduced the dissemination of the virus in the viraemic phase and consequently eliminated the opportunity for neural spread. However, as shown by our quick transport experiments, inoculation of the glyE$^-$ mutant in a high dose was not sufficient for the neuritic uptake and axonal transport, which occurred with the parental ANGpath variant as with the ANG strain directly upon the high virus input.

2. The glyC$^-$ ANGpathgC18 mutant showed a limited but clear-cut replication at the portal of entry, in the spleen, adrenal gland and minimal spread by the bloodstream. Minimal invasion of the peripheral and vegetative nervous system was detected. However, the neuritic uptake and quick axonal spread could not be followed by oral corneal inoculation, since the virus input of 2×10^7 PFU was not sufficient for coming into an efficient contact with the nerve endings (as evidenced by the events after the inoculation of the lower ANGpath dose of 8×10^5 PFU). Mannini-Palenzona *et al.* (1988) found no restriction in the capacity of the glyC minus mutant of HFEM strain to establish latency. Johnson *et al.* (1986) found no restriction of virus spread with an HSV-2 glyC minus mutant inoculated by intravaginal route. These results were confirmed also in our experiments (Rajčáni et al., 1990).

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