J. gen. Virol. (1983), **64**, 1973–1982. *Printed in Great Britain*

Key words: *HSV-1/neuropathogenicity/protection/host defence*

Neuropathogenicity of Herpes Simplex Virus in Mice: Protection against Lethal Encephalitis by Co-infection with a Non-encephalitogenic Strain

By C. H. SCHRÖDER,* G. KÜMEL, J. GLORIOSO,[1] H. KIRCHNER AND H. C. KAERNER

Institute for Virus Research, German Cancer Research Center, Heidelberg, Federal Republic of Germany and [1]Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109, U.S.A.

(*Accepted 31 May 1983*)

SUMMARY

Intraperitoneal infection of susceptible mice with an apathogenic herpes simplex virus type 1 (HSV-1) strain prevented the lethal outcome of a challenge infection with a pathogenic strain, even if the challenge preceded the protective infection. It was found that the protective inoculation blocks the initial replication of the challenge virus. In addition, intraperitoneal infection with the protective HSV-1 strain led to the induction of a refractory state in the central nervous system, resulting in resistance to direct intracranial infection with HSV-1. This state is also inducible locally by intracerebral inoculation of a non-replicating mutant virus. The results indicate that HSV-1 strains differing in neurovirulence may differ in the induction or the sensitivity to this protective effect. Experiments with non-replicating HSV-1 temperature-sensitive strains demonstrated that protection against lethal infection does not depend on replication or expression of late genes of the protective strain. Inoculation of animals with detergent-soluble extracts of infected cells or infected and u.v.-irradiated syngeneic cells protected the animals against co-infection with encephalitogenic challenge virus. The experiments define this protective effect as an antigen-induced immediate host defence mechanism active within 24 h post-infection.

INTRODUCTION

Experimental infections of mice have been used in many studies as model systems to elucidate the mechanisms of pathogenicity of herpes simplex virus (Andervont, 1927; Catalano *et al.*, 1970; Zisman *et al.*, 1970; Stevens & Cook, 1971; Lopez, 1975; Kirchner *et al.*, 1978). Recently, we have described the spread of infection, induction of host defence functions, neurovirulence and latency characteristics of herpes simplex virus type 1 (HSV-1) ANG. This virus replicates to high titres in mouse organs after systemic or peripheral infection without causing signs of neurological disease or death of the animals (Kümel *et al.*, 1982). The pattern of infection of this strain has been contrasted with that of the neurovirulent strains HSV-1 WAL and HSV-1 ANG path, the latter being an intrastrain variant of HSV-1 ANG with very minor genome alterations (Kaerner *et al.*, 1981). This variant, however, replicates in the brain within 3 to 4 days after infection, leading to lethal encephalitis (Kümel *et al.*, 1982).

Several mechanisms can be excluded as possible causes for differences in the pathogenicity of individual HSV-1 strains. Pathogenic and apathogenic virus strains initially replicate at the site of infection and establish a virus reservoir in the spleen, leading to a masked and ineffective viraemia and the elimination of virus within 5 days post-infection from the visceral organs of the mouse (Kümel *et al.*, 1982; Kaerner *et al.*, 1983). In addition, both the non-encephalitogenic HSV-1 ANG strain and its encephalitogenic variant HSV-1 ANG path are thymidine kinase-

* Mailing address: Institut für Virusforschung, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg 1, F.R.G.

0022-1317/83/0000-5703 $02.00

positive, and show similar growth properties *in vitro*. Both HSV-1 strains were lethal in immunoincompetent newborns and in animals immunosuppressed with cyclophosphamide. The resistance of thymusless nude mice against HSV-1 ANG infection suggested that the IgG response and mature T lymphocytes do not play a role in the observed difference in neurovirulence (Kümel *et al.*, 1982). The HSV-specified function determining the difference in neurovirulence between encephalitogenic and non-encephalitogenic HSV-1 strains apparently is effective before the third day post-infection.

Recently, we described a virus-induced defence phenomenon effectively inhibiting transport to, or replication in, the CNS of the otherwise lethally neurovirulent HSV-1 strain WAL by simultaneous intraperitoneal (i.p.) infection with strain ANG (Schröder *et al.*, 1981). This protection phenomenon could be demonstrated also with a less sensitive mouse strain C57BL/6 or by co-infection of DBA/2 mice with HSV-1 ANG and pathogenic HSV-1 isolates other than HSV-1 WAL (Kaerner *et al.*, 1983). In this study the virus-induced host defence process with the above features was further investigated. The data obtained lead to the conclusion that peripheral infection with a non-encephalitogenic strain induces a protective principle in the central nervous system (CNS). This protection depends on the quantity and activity of virus-specific products presented to the host organism and may explain the difference between encephalitogenic and non-encephalitogenic HSV strains.

METHODS

Mice. Inbred mouse strains DBA/2 and BALB/c were purchased from Deutsche Gesellschaft für Versuchstierkunde (Hannover, F.R.G.). Young adult mice, 6 to 8 weeks old, were used for all experiments.

Infection mode and dissection methods. Male DBA/2 or BALB/c mice were inoculated i.p. and/or intracerebrally (i.c.) with live virus contained in 0·2 ml or in 0·02 ml phosphate-buffered saline (PBS) respectively as described previously (Kümel *et al.*, 1982). The method for dissection of organs has been described (Kümel *et al.*, 1982). Dissected organs were disintegrated by ultrasonic treatment and by freezing and thawing and then suspended in Eagle's minimal essential medium (MEM) containing 100 mg/l gentamicin and the anti-mycotic drug amphotericin B (2·5 mg/l). Determination of the virus content in mouse organs was done by independent parallel titrations of sonicated tissue samples from three to five mice.

Cells and virus. HSV-1 strains were routinely passaged at low multiplicity of infection (0·03 p.f.u./cell) in an African green monkey kidney cell line (RC-37 Rita; Italdiagnostics, Rome, Italy) as described previously (Schröder *et al.*, 1975/76). Cells were grown in Eagle's MEM containing 7% foetal calf serum. Virus titres were determined as described by Russell (1962). The origins and characterization of the HSV-1 strains ANG and WAL and the ANG mutant *ts*7 have been described previously (Darai & Munk, 1976; Schröder *et al.*, 1975/76; Müller *et al.*, 1980); HSV-1 strain KOS and the temperature-sensitive mutant HSV-1 KOS *ts*J12 (Little *et al.*, 1981) were kindly provided by P. A. Schaffer. 3T3 cells (American Type Culture Collection; CRL 163) were grown in Dulbecco's MEM containing 7% foetal calf serum.

Antigen preparations. Monolayers of 3T3 cells in 145 mm dishes were infected at an m.o.i. of 5 for 24 h and infectious virus was eliminated by u.v.-irradiation (8 J/m^2/min for 20 min). The irradiated cells were scraped off the plastic surface and suspended in PBS. Titration showed that the antigen suspensions contained less than 5 p.f.u./ml of live virus.

For the preparation of detergent-soluble extract (DSE), RC-37 cells were infected at an m.o.i. of 2 with HSV-1 and harvested 24 h post-infection. Detergent-soluble extracts of these cells were prepared using Nonidet P40 as described by Lesnik & Ross (1975).

RESULTS

Intraperitoneal co-infection with a non-pathogenic HSV-1 strain leads to protection of susceptible mice against lethal HSV-1 infection

Systemic or peripheral infection of highly susceptible mice (DBA/2) with HSV-1 strain ANG did not kill the animals even at the highest virus doses (Table 1) whereas, as shown earlier, intracranial application of only 1 p.f.u. of this virus results in lethal encephalitis (Kümel *et al.*, 1982). HSV-1 strain WAL, however, causes lethal encephalitis in the same mouse strain after i.p. infection, with an LD_{50} of 2×10^2 p.f.u. (Engler *et al.*, 1982). This fatal outcome of the infection with HSV-1 WAL could, however, be overcome by simultaneous infection with HSV-1 ANG (Table 1). Similar results were obtained using the BALB/c mouse strain (data not shown).

Table 1. *Protection of DBA/2 mice against challenge virus infection by simultaneous infection with HSV-1 ANG or HSV-1 KOS**

Protection		Challenge		
HSV-1 strain	i.p. dose	HSV-1 strain	i.p. dose	Dead mice/group
–	–	ANG	2×10^7	0/10
–	–	ANG path	1×10^3	10/10
–	–	WAL	1×10^3	10/10
–	–	KOS	3×10^6	0/12
ANG	1×10^5	WAL	1×10^3	2/20
ANG	2×10^6	WAL	1×10^3	1/20
ANG	1×10^5	ANG path	1×10^3	1/20
KOS	1×10^5	WAL	1×10^3	0/10

* In each group 6- to 8-week-old DBA/2 mice were injected intraperitoneally with 0·2 ml of saline containing the indicated virus dose. In simultaneous co-infection experiments, challenge infection with the lethal strains was carried out 2 h post-infection with the protecting HSV-1 strain ANG or KOS.

Table 2. *The protection phenomenon depends on the relative timing of intraperitoneal infection with HSV-1 ANG and HSV-1 WAL*

Time of challenge*	Dead mice/group
8 h before infection	9/10
4 h before infection	3/10
0 h before infection	2/10
2 h post-infection	1/10
24 h post-infection	0/10
2 days post-infection	1/10
3 days post-infection	0/10
4 days post-infection	0/10
7 days post-infection	0/10

* Groups of ten DBA/2 mice were infected with 2×10^5 p.f.u. HSV-1 ANG and superinfected with 10^3 p.f.u. HSV-1 WAL at various times before or after the protective infection. In a control group that received 10^3 p.f.u. HSV-1 WAL alone, 10/10 animals died.

Table 1 further shows that i.p. infection with HSV-1 ANG protected DBA/2 mice also against the sequelae of infection with HSV-1 ANG path. The induction of protection by another apathogenic strain of HSV-1, strain KOS, is included in Table 1 to show that this quality did not correlate with the capacity to form syncytia. Strain ANG is of the syn phenotype while strain KOS is syn$^+$. A correlation between lack of neurovirulence and the ability to form syncytia was not observed (Dix & Baringer, 1981). Moreover, these results show that the protective effect is not strain-dependent.

The protection afforded by the administration of apathogenic virus with respect to the relative timing of infection with the pathogenic virus is shown in Table 2. Protection was observed even when the apathogenic virus was injected 4 h after injection of the pathogenic virus. To characterize the protection phenomenon in more detail, the spread of strain ANG and WAL in the course of infection was determined. The plaques induced by these strains may be differentiated, since HSV-1 WAL is syn$^+$. Fig. 1 shows the kinetics of virus spread after i.p. infection of DBA/2 mice with the strain ANG alone (*a*), with the strain WAL alone (*b*) or after infection with both strains at a time interval of 2 h (*c*). It can be seen from Fig. 1 that infection with an excess of HSV-1 ANG over HSV-1 WAL effectively restricted the primary replication of the WAL strain in the peritoneal cavity and its spread to the spleen and the CNS. Protection by i.p. co-inoculation of non-pathogenic virus results in blocking primary replication of the challenge virus.

This restriction in the replication and spread of the virus was overcome by increasing the dose of the pathogenic challenge virus. Table 3 shows that after i.p. infection with both strains at the same dose, both viruses replicated to high titres in the peritoneum, spread to the spleen and the

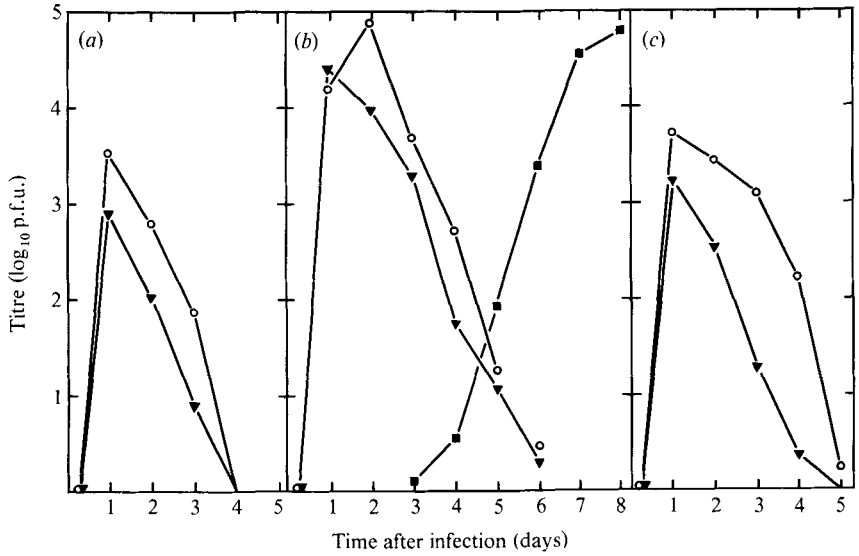

Fig. 1. The kinetics of virus spread after i.p. infection of DBA/2 mice with HSV-1 strain ANG (*a*), WAL (*b*) or after infection with both strains (*c*). Groups of 30 DBA/2 mice were infected with 2×10^5 p.f.u. HSV-1 ANG, 10^3 p.f.u. HSV-1 WAL or both in a time interval of 2 h. At appropriate time points, groups of three to six mice were sacrificed and dissected. Virus content in mouse organs was determined by independent titration of samples of sonicated tissue: peritoneal exudate (▼), spleen (○) and brain (■). Mice not sacrificed in the experiment were observed for signs of neurological disease. All of these mice infected with strain WAL alone developed lethal encephalitis, whereas all of the other groups survived 20 days without signs of illness. In animals infected with both strains, only virus of the syn phenotype (ANG) could be detected and no virus at all in the brain during the sampling period of 8 days post-infection.

Table 3. *Virus spread after intraperitoneal co-infection with equal doses of the HSV-1 strains ANG (syn) and WAL (syn⁺)*

Organ	Plaque type	Virus yield (p.f.u.)* 24 h	48 h	6 days
PE	syn	2×10^3	4×10^4	ND†
	syn⁺	6×10^3	2×10^6	
Spleen	syn	10	4×10^2	20
	syn⁺	0	3×10^3	0
Brain	syn	0	0	0
	syn⁺	0	0	6×10^4

* Virus yield at the times shown after simultaneous co-infection with 2×10^5 p.f.u. each of HSV-1 strains ANG and WAL. All values are the mean of the virus yield of the respective mouse organs of three mice or the peritoneal exudate (PE), obtained by independent titration of samples.

† ND, Not done.

infection led to lethal encephalitis (10/10 animals). However, only syn⁺ plaques, corresponding to the neurovirulent strain WAL, could be demonstrated in titrations of progeny virus from the brain. This observation applies as well to co-infection with other apathogenic HSV-1 strains together with neurovirulent strains other than WAL (data not shown).

Primary replication of the protecting virus is not a prerequisite for the protection phenomenon

The results in Fig. 1 (*c*) indicated that the protective virus replicated actively under the conditions of the experiment. The DNA-negative, temperature-sensitive mutant *ts*7 derived from HSV-1 ANG was used to test whether this primary virus replication was required for

Table 4. *Protection against intraperitoneal infection with HSV-1 WAL by HSV-1 ANG* ts*7 and the lack of virus spread*

Organ	Plaque type	Virus yield (p.f.u.)* 24 h	48 h	6 days
PE	syn	15†	0	0
	syn$^+$	0(3×10^3)‡	0(2×10^2)	0
Spleen	syn	10	0	0
	syn$^+$	0(2.5×10^2)	0(4×10^3)	0
Brain	syn	0	0	0
	syn$^+$	0	0	0(3×10^3)

* Virus yield at the times shown after simultaneous intraperitoneal infection with 10^6 p.f.u. HSV-1 ANG *ts*7 and 10^3 p.f.u. HSV-1 WAL.

† All values are the mean of the virus yield of the respective organs or the peritoneal exudate (PE) of three mice, obtained by independent titration of samples.

‡ Values in parentheses designate virus in the organs of mice which had been infected with 10^3 p.f.u. HSV-1 WAL alone as a control.

Table 5. *Intravenous and intracerebral challenge infection with HSV-1 WAL of mice protected by HSV-1 ANG**

Challenge virus: Route of infection	Days after infection	Dead mice/group
Intravenous	0	3/10
	1	1/10
	2	1/10
Intracerebral	0	10/10
	1	10/10
	2	4/10
	3	1/10
	4	1/10
	5	0/10

* Groups of ten DBA/2 mice were infected i.p. with 2×10^5 p.f.u. HSV-1 ANG and superinfected at the time points indicated with 2×10^3 p.f.u. intravenously or 2×10^2 p.f.u. i.c. of HSV-1 WAL.

protection. The results of the experiments demonstrate that the *ts* mutant did not replicate in mouse organs. It was, however, capable of blocking the replication of the challenge virus (Table 4) and induced a protective response: in mice co-infected with the mutant *ts*7 and HSV-1 WAL, only one out of ten animals died. In a control group infected with HSV-1 WAL (ten animals) all of the mice died, while the HSV-1 ANG mutant *ts*7 proved to be apathogenic. Therefore, the protection phenomenon did not depend on primary replication of the protecting virus at the site of infection. From the results so far, the protection can be explained at the level of virus–cell interaction in the peritoneum.

The protection phenomenon involves a state of unresponsiveness in the CNS by the induction of host defence processes

Although the apathogenic HSV-1 strain ANG was capable of blocking the replication of other HSV strains at the site of primary replication in the peritoneum, it was unclear whether protection against encephalitis resulted from an additional prevention of the replication of HSV-1 virus in the nervous system.

Consequently, we tested whether i.p. infection with the apathogenic HSV-1 strain ANG protected the experimental animal against infection with the challenge virus at other injection sites. Infection leading to encephalitis might involve movement of the virus to the CNS by haematogenous or axonal transport. Accordingly, we examined the protective effect after protective i.p. infection with HSV-1 ANG by a challenge infection with HSV-1 WAL (i) intravenously or (ii) directly into the brain, i.c. Table 5 shows the results of these experiments. In

Table 6. *Survival time after intracerebral challenge with HSV-1 WAL*

Protective infection i.p. (HSV-1 ANG) dose (p.f.u.)	Challenge infection i.c. (HSV-1 WAL) Dose (p.f.u.)	Days post-infection	Survival time*	Dead mice/group
4×10^5	–	–		0/10
None	50	–	4·6	10/10
4×10^5	50	0	4·5	10/10
	50	1	5·4	10/10
	50	2	7·0	4/10
	50	3	–	1/10

* Mean time of survival (days) after challenge infection with HSV-1 WAL.

Table 7. *Protection of DBA/2 mice by i.p. infection with HSV-1 ANG or the mutant strain ANG* ts7 *against an intracerebral challenge infection with the same strain, a pathogenic variant of HSV-1 ANG, or HSV-1 WAL at various doses*

Protective infection (i.p.) HSV-1 strain	Dose (p.f.u.)	Challenge infection (i.c.) HSV-1 strain	Dose (p.f.u.)	Dead mice/group
ANG	4×10^5	ANG	500	0/10
			5000	5/10
		ANG path	5	0/10
			50	2/10
			500	10/10
		WAL	50	1/10
			500	8/10
			5000	10/10
ANG *ts*7	4×10^5	WAL	50	0/10
			500	1/10
			5000	8/10
None	–	ANG	1	10/10
			10	10/10
		ANG path	1	10/10
			10	10/10
		WAL	1	10/10
			10	10/10

the bypass of the presumptive transition from the peritoneum to the blood system by intravenous injection, the challenge did not escape the protective effect of HSV-1 ANG. Even more important was the finding that HSV-1 ANG induced in the CNS a state refractory to i.c. infection with HSV-1 WAL within 48 h after infection (Table 5). Table 6 shows that the time of survival is considerably prolonged under these conditions and indicates that protection takes effect in the CNS as early as 24 h after i.p. infection with the protective strain.

The fact that i.p. infection with HSV-1 ANG protected against i.c. challenge with HSV-1 WAL supports the hypothesis that the lack of pathogenicity of HSV-1 ANG might be explained by its protective capacity. The data in Table 7 demonstrate that systemic infection with HSV-1 ANG indeed induced the CNS to be refractory against direct i.c. inoculation of otherwise lethal doses of this same virus, HSV-1 ANG. This applies also to i.c. challenge with the pathogenic derivative of HSV-1 ANG. The temperature-sensitive mutant HSV-1 ANG *ts*7 did not replicate in the peritoneum (Table 4) and was also able to protect the mice against the sequelae of i.c. infection with HSV (Table 7). It could be concluded that protection is effected by a host defence system. Apparently, the mere presence of the virus particles in the absence of active replication induced the refractory state in the CNS.

Table 8. *Protection of DBA/2 mice by intracerebral inoculation of the i.c. non-lethal mutant HSV-1 KOS* ts*J12 against subsequent intracerebral challenge infection with HSV-1 WAL at various times after protective infection*

Protective infection i.c.		Challenge infection i.c.			
Virus	Dose (p.f.u.)	Virus	Dose (p.f.u.)	Time (h) after protective infection	Dead mice/group
HSV-1 KOS *ts*J12	4×10^5	–	–	–	0/12
		HSV-1 WAL	10^3	2	5/5
			10^3	24	4/5
			10^3	48	3/10
			10^3	72	0/10
–	–	HSV-1 WAL	10^1	–	5/5

The state of unresponsiveness in the CNS can be induced by intracerebral application of virus

To characterize further the protection phenomenon, we tested whether protection was induced by injection of virus directly at the site where it becomes effective, that is by direct i.c. injection of both protective and challenge virus. Since the virus strains described as 'non-pathogenic' in DBA/2 mice, like HSV-1 ANG or HSV-1 KOS, all lead to lethal neurological disease if injected intracerebrally, we had to choose a mutant that did not replicate as protective virus. Despite the fact that HSV-1 ANG *ts*7 does not replicate in the peritoneum, i.c. inoculation of this virus led to lethal encephalitis of the animals even at very low doses. The virus produced in the brain proved to be temperature-sensitive *in vitro*.

The mutant HSV-1 KOS *ts*J12 undergoes only one round of replication at non-permissive temperature, resulting in penetration-negative progeny virus lacking glycoprotein B (Little *et al.*, 1981). Therefore, we tested this virus for induction of protection following i.c. injection. Table 8 shows that i.c. injection with *ts*J12 virus did not lead to lethal encephalitis even at doses of 4×10^6 p.f.u. In a control experiment it could be demonstrated that after i.c. inoculation, replication of HSV-1 KOS *ts*J12 was indeed not detectable. Intracerebral injection of challenge virus at various intervals after i.c. injection of *ts*J12 virus showed that the mutant is able to induce a refractory state in the brain after 2 days (Table 8).

In a parallel experiment the mice were injected i.c. with particle doses similar to those above, but with *ts*J12 virus grown at 39 °C. This virus preparation was unable to infect cells in culture and did not protect the animals against i.c. challenge virus injection (data not shown).

Protection is induced by virus-specific protein in the absence of live virus

The data obtained with the temperature-sensitive mutants HSV-1 ANG *ts*7 and HSV-1 KOS *ts*J12 indicated that viral antigens alone might be capable of inducing protection. To support this conclusion and to determine whether the neuropathogenicity of strains ANG path and WAL was associated with their inability to induce protection, u.v.-irradiated 3T3 cells that had been infected with HSV were injected into the peritoneum of BALB/c mice. Protection against subsequent i.p. challenge with the pathogenic HSV-1 WAL was obtained regardless of whether the apathogenic strain HSV-1 KOS or the pathogenic strain HSV-1 WAL was used for the infection of 3T3 cells (Table 9). The fact that no protection was observed following injection of uninfected cells indicates the specificity of the protective response (Table 9).

Detergent extracts of RC-37 cells that had been infected with HSV-1 strains ANG, ANG path, KOS or WAL also proved protective for BALB/c mice against i.p. challenge infection with otherwise lethal doses of HSV-1 WAL. Extracts from uninfected cells did not induce protection (Table 9). Thus, antigen preparations carrying viral antigens but no infectious virus lead to the immediate form of protection regardless of whether the antigens are derived from an encephalitogenic or non-encephalitogenic HSV-1 strain.

Table 9. *Protective capacity of virus-specific products in the absence of live virus**

Protective agent	Infection of cells	Dead mice/group
None	–	8/8
3T3 cells	Mock infection	8/8
	KOS	0/7
	WAL	1/7
DSE	Mock infection	8/8
	ANG	2/10
	ANG path	1/7
	KOS	2/7
	WAL	1/7

* Groups of BALB/c mice were injected i.p. with 3T3 cells or detergent-soluble extract (DSE) from RC-37 cells. The cells had been mock-infected or infected, irradiated and tested for residual live virus as described in Methods. All animals were challenged i.p. with 10^5 p.f.u. of HSV-1 WAL 24 h later. Additional control groups that had received antigen treatment but no challenge survived 21 days without signs of illness.

DISCUSSION

Non-encephalitogenic HSV-1 strains can protect mice from synchronous challenge with pathogenic strains. This phenomenon could be explained by demonstrating that systemic infection with the non-neurovirulent strain HSV-1 ANG was able to restrict the course of infection by strain WAL at a very early stage, namely by blocking the initial replication at the primary site of infection. Considering the large excess of protective over challenge virus, this explanation does not necessarily involve host defence mechanisms; the phenomenon can be understood at the level of virus–cell interaction. A role for defective interfering particles is excluded since the virus strains used were routinely passaged at low m.o.i. (Schröder & Urbaczka, 1978). As reported earlier, differences in the induction of interferon and sensitivity to interferon could be excluded in a comparative study of HSV-1 ANG and HSV-1 WAL (Kümel *et al.*, 1982). No experimental basis was found for a discriminatory role of natural killer cell induction or for differential replication in macrophages (Engler *et al.*, 1981; Kümel *et al.*, 1982).

The protective mechanism becomes effective even in the absence of virus replication or expression of late gene products, and it is induced by antigen preparations that are free of live virus. Protection is observed after simultaneous infection of a pathogenic and an apathogenic virus strain and is most effective if time is allowed for the apathogenic strain to adsorb to and replicate in the peritoneal target cells. Protection, however, is still induced even if the challenge virus is injected 4 h prior to the protective virus; this excludes competition for receptor sites as a possible mechanism. This time pattern contrasts with that of known forms of host defence provided by a virus-induced immune response.

The lack of protection and the concomitant replication of challenge virus after synchronous i.p. infection of equal doses of protective and challenge virus implies that a block to the replication of challenge virus at the site of primary infection is one of the principal factors in protection. Strain ANG on the other hand is still not able to reach the CNS and to replicate in the CNS. This result suggests that the inability of HSV-1 to reach the CNS is due to a specific property of this virus and to the induction of an active principle discriminating between the two strains upon i.p. infection with equal virus doses.

Therefore, we tested the capacity of HSV-1 ANG to induce a protective process in addition to the restriction of initial replication discussed above, which could be tentatively described in terms of virus–cell interaction and interference between the two HSV-1 virus strains. Challenge virus was injected into body sites where a direct interaction of the two viruses was not to be expected. As shown previously (Kümel *et al.*, 1982), after i.p. infection with HSV-1 ANG very little virus can be found in the blood and none at all in the brain. Consequently, challenge virus was injected intravenously or i.c. into mice previously i.p. infected with the protective strains. These experiments revealed that infection with non-pathogenic virus renders the animal insensitive to an otherwise lethal intracranial infection with more than 5×10^3 LD_{50} (for i.c. infection) and induces in the brain a state in which it is refractory to HSV infection. Kinetic

experiments demonstrated that this protective principle is induced within 1 to 3 days after infection.

This type of experiment shows the initiation of a protective principle in the CNS by systemic infection with HSV within the surprisingly short time of 24 to 72 h. Magrassi (1935) found that peripheral injection of rabbits protected the animals against i.c. challenge with the same virus, but the protective effect was seen only on day 7 post-infection and was not observed with shorter or longer time intervals between the two inoculations. This phenomenon, as yet not understood, was the subject of the study by Catalano *et al.* (1970) in mice. They found a rather slight protection 6 days after primary infection with low dóses of pathogenic viruses. Our results with early i.c. challenge are consistent with the hypothesis that the essential difference between a non-neurovirulent HSV-1 strain like ANG and neurovirulent strains like WAL or ANG path is the capacity to induce such a refractory state in the CNS.

Haematogenous or axonal transport of virus to the CNS have been discussed as possible routes of infection with HSV. Considering the data indicating that very little virus can be found in the blood of infected mice (Kümel *et al.*, 1982), the spread of virus to the CNS should be a rare event. The extremely high susceptibility to i.c. infection would explain the deleterious effect of such a rare process. Accordingly, the difference between HSV strains in their neurovirulence could then be attributed to the sensitivity to the protective process induced in the CNS, or in the capacity to overcome this process by a rapid transport of a higher number of virus particles to the brain.

Several arguments can be derived from the findings that i.c. infection with the HSV-1 KOS mutant *ts*J12 does induce a protective principle while i.c. infection with the adsorption-negative progeny of this virus fails to protect the animals. Since *ts*J12 undergoes only one round of replication, it can be assumed to become effective by locally induced processes. Possibly excretion, or exhibition at cell surfaces, of virus-induced antigens in the brain might be a necessary condition for protection to be induced. However, intravenous dissemination of i.c. infected virus cannot be excluded (Mims, 1960).

The possibility that viral proteins alone could lead to early protection was verified by the finding that infected irradiated mouse 3T3 cells or even detergent extracts from infected cells protect the animals against i.p. or i.c. challenge. In addition, these experiments revealed that antigen preparations derived from encephalitogenic HSV-1 strains protect the animals. This finding implies that neuropathogenicity of HSV-1 strains is due to a dynamic relationship between the amount and activity of viral antigen on one hand and the growth and spread of the virus on the other. The time schedule of the protection phenomenon does not suggest a conventional immunological explanation. As to a possible mechanism, one nevertheless is led to speculate on a very early immune response possibly restricted to the CNS.

This work was supported by the Deutsche Forschungsgemeinschaft (Project Ka 372/2-1). J. Glorioso was supported by Public Health Service Grant NIH RR00200 from the Animal Resource Branch, DRR, by NIH Grants AI 17900 and AI 18228 and by the German Cancer Research Center, Heidelberg, F.R.G. We thank Mr D. Baumgartl for his cooperation in the animal experiments.

REFERENCES

ANDERVONT, H. B. (1927). Activity of herpetic virus in mice. *American Journal of Hygiene* **14**, 383–393.

CATALANO, L. W., MOOSSY, J. & SELL, S. (1970). Viral interference in the brains of mice infected subcutaneously with herpes simplex virus. *Proceedings of the Society for Experimental Biology and Medicine* **135**, 509–514.

DARAI, G. & MUNK, K. (1976). Neoplastic transformation of rat embryo cells with herpes simplex virus. *International Journal of Cancer* **18**, 469–481.

DIX, R. D. & BARINGER, J. R. (1981). Comparative neurovirulence of syncytiogenic and nonsyncytiogenic herpes simplex virus type 1 (HSV-1) strains. In *International Workshop on Herpes Viruses*, p. 161. Edited by A. S. Kaplan, M. LaPlaca, F. Rapp & B. Roizman. Bologna: Esculapio Publishing Co.

ENGLER, H., ZAWATZKY, R., GOLDBACH, A., SCHRÖDER, C. H., WEYAND, C., HÄMMERLING, G. J. & KIRCHNER, H. (1981). Experimental infection of inbred mice with herpes simplex virus. II. Interferon production and activation of natural killer cells in the peritoneal exudate. *Journal of General Virology* **55**, 25–30.

KAERNER, H. C., BAUMGARTL, D., ZELLER, H., SCHATTEN, R. & OTT-HARTMANN, A. (1981). Peripheral pathogenicity in mice acquired by an originally non-pathogenic strain of herpes simplex virus after serial passages in mouse brain. In *International Workshop on Herpes Viruses*, pp. 151–152. Edited by A. S. Kaplan, M. LaPlaca, F. Rapp & B. Roizman. Bologna: Esculapio Publishing Co.

KAERNER, H. C., SCHRÖDER, C. H., OTT-HARTMANN, A., KÜMEL, G. & KIRCHNER, H. (1983). Genetic variability of herpes simplex virus (HSV) type 1: development of a pathogenic variant from an apathogenic strain of HSV-1 in mouse brain. *Journal of Virology* **46**, 83–93.

KIRCHNER, H., HIRT, H. M., KOCHEN, M. & MUNK, K. (1978). Immunological studies of HSV infection of resistant and susceptible inbred strains of mice. *Zeitschrift für Immunitätsforschung und experimentelle Therapie* **154**, 147–154.

KÜMEL, G., KIRCHNER, H., ZAWATZKY, R., ENGLER, H., SCHRÖDER, C. H. & KAERNER, H. C. (1982). Experimental infection of inbred mice with herpes simplex virus. V. Investigations with a virus strain non-lethal after peripheral infection. *Journal of General Virology* **63**, 315–323.

LESNIK, F. & ROSS. L. J. M. (1975). Immunization against Marek's disease using Marek's disease virus-specific antigens free from infectious virus. *International Journal of Cancer* **16**, 153–163.

LITTLE, S. P., JOFRE, J. T., COURTNEY, R. J. & SCHAFFER, P. A. (1981). A virion-associated glycoprotein essential for infectivity of herpes simplex virus type 1. *Virology* **115**, 149–160.

LOPEZ, C. (1975). Genetics of natural resistance to herpesvirus infections in mice. *Nature, London* **258**, 152–153.

MAGRASSI, F. (1935). Studii sull'infezione e sull'immunita da virus erpetico. *Zeitschrift für Hygiene und Infektionskrankheiten* **117**, 573–584.

MIMS, C. A. (1960). Intracerebral injections and the growth of viruses in the mouse brain. *British Journal of Experimental Pathology* **41**, 52–59.

MÜLLER, U., SCHRÖDER, C. H., ZENTGRAF, H. & FRANKE, W. W. (1980). Coexistence of nucleosomal and various non-nucleosomal chromatin configurations in cells infected with herpes simplex virus. *European Journal of Cell Biology* **23**, 197–203.

RUSSELL, W. C. (1962). A sensitive and precise plaque assay for herpes virus. *Nature, London* **195**, 1028–1029.

SCHRÖDER, C. H. & URBACZKA, G. (1978). Excess of interfering over infectious particles in herpes simplex virus passaged at high m.o.i. and their effect on single-cell survival. *Journal of General Virology* **41**, 493–501.

SCHRÖDER, C. H., STEGMANN, B., LAUPPE, H. F. & KAERNER, H. C. (1975/6). An unusual defective genotype derived from herpes simplex virus ANG. *Intervirology* **5**, 173–184.

SCHRÖDER, C. H., ENGLER, H. & KIRCHNER, H. (1981). Protection of mice by an apathogenic strain of HSV-1 against lethal infection by a pathogenic strain. *Journal of General Virology* **52**, 159–161.

STEVENS, J. G. & COOK, M. L. (1971). Restriction of herpes simplex virus by macrophages. An analysis of the cell–virus interaction. *Journal of Experimental Medicine* **133**, 19–38.

ZISMAN, B., HIRSCH, M. S. & ALLISON, A. C. (1970). Selective effects of antimacrophage serum, silica and anti-lymphocyte serum on pathogenesis of herpes virus infection of young adult mice. *Journal of Immunology* **104**, 1155–1159.

(Received 31 March 1982)