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THE CONTINUING PROBLEM OF HERPES SIMPLEX VIRUS PERSISTENCE*

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Summary. — While the main interest in the pathogenesis of herpes simplex virus (HSV) in the sixties had been focussed on acute infections, in the seventies latent infection has become the main goal of investigation. Despite of overwhelming literature, the HSV persistence has remained a continuing problem from the practical as well as theoretical points of view. Nevertheless, the following conclusions can be made: 1) HSV spreads along nerves inside as well as outside axons; 2) it resides in a non-productive form for lifelong in the sensory or vegetative ganglia; and 3) it is intermittently activated when causing peripheral virus shedding or recurrent disease. The persistence of HSV DNA in neurons may be associated with a limited transcription and translation, but the ganglia in a great majority of subjects are uninfectious during the latency period.

Key words: *herpes simplex virus*; *latent infection*; *experimental animals*

Experimental animals (mouse, rabbits, guinea pigs) have turned out useful for investigations on pathogenesis of HSV infections. These animal models, according to their age and the virus dose administered, roughly correspond to the outcome of virus-host interactions in man: an acute (sometimes lethal) disease or a prolonged carriership (latency) with or without occasional (intermittent) virus shedding.

The acute disease

Acute lethal disease develops in newborn mice regardless of the inoculation route. In suckling mice viraemia often occurs after intraperitoneal (i.p.) inoculation, the virus being disseminated through the body by macrophages. In contrast, the threshold dose causing transient viraemia and encephalomyelitis in adult outbred mice is at least 1000 times higher (Rajčáni *et al.*, 1970). This can be explained by increased resistance of macrophages due to their age-dependent maturation. Virus assembly and release do not occur in the macrophages of adult outbred mice, although virus DNA synthesis and proteosynthesis in them are unaltered (Stevens, Cook, 1971).

* Dedicated to Prof. D. Blaškovič on his 70th birthday

Genetic resistance of C3H/HeJ mice after peripheral HSV administration is mediated by macrophages, since these mice are equally susceptible to intracerebral (i.c.) inoculation of HSV (Kirchner *et al.*, 1978*a*). Only 3 out of 8 virus strains multiplied in NMRI macrophages and none except one had grown in unstimulated macrophages from resistant C57/bl mice (Frank *et al.*, 1978). In primoinfection, macrophages are very early effective after virus entry; later on interferon, natural killer cells, the humoral and cellular immune responses are gradually involved, the latter being responsible mainly for the recovery from disease (Rouse and Babiuk, 1978). As recently shown, the resistance of inbred mice can be impaired also by silica, bone marrow irradiation and carrageenan; it is mediated by the bone marrow-dependent and thymus independent non-adherent mononuclear NK cells (Lopez, 1981). Adoptive transfer of spleen cells coming from donors infected 4—5 days previously with HSV-2 efficiently reduces virus multiplication in the liver of syngeneic (BALB/c) recipients, when performed by 24 hr after i.p. inoculation of the virus (Mogensen, 1982). Cyclophosphamide or its metabolite acts on both adherent and non-adherent mononuclear leukocytes; therefore, it effectively enhances the host's susceptibility to the virus not only facilitating the neural spred but also the generalization of HSV via bloodstream (Rajčáni *et al.*, 1974).

Neural spread

Although the blood leukocytes may carry the virus to various tissues, the dominating pathway of HSV transmission is the neural spread. This can be prevented only before the virus had attached to nerve endings. In newborn, weanling as well as in adult mice HSV reaches the CNS along cerebrospinal and vegetative nerves (Lascano *et al.*, 1980). The Schwann cells of nerve trunks are permissive in highly susceptible hosts (Rajčáni, Conen, 1972), but they are semipermissive in less susceptible ones (Cook, Stevens, 1973). When endoneural space of the nerve root is overwhelmed with virions, the highly permissive astrocytes at the nerve entrance zone become infected (Townsend, Baringer, 1978). HSV can get access to the astrocytes also by crossing the blood-brain barrier as the vascular endothelium contains pinocytic vesicles which may carry virus particles (Yamamoto *et al.*, 1973). The astrocytes as well as the nerve endings are rich of HSV-receptors which are not present on the neuronal body (Vahlne *et al.*, 1978).

Considering that the epithelium of mucous membranes represents the only mechanical barrier preventing virus attachment to nerve endings, one understands why neural spread plays a crucial role in the HSV pathogenesis. Resident macrophages fail to prevent virus attachment in non-immune mice inoculated with 10^5—10^6 PFU of HSV. Once attached to nerve endings, the virions are quickly engulfed and transported within axons. The adsorption to nerve endings occurs within 60—90 min (Field *et al.*, 1975), the axonal transport is as quick as 1.5 mm/hr. The quick intraaxonal transport can be blocked by vinblastin or colchicin which impair the polymerization of tubulin (Kristensson *et al.*, 1971). The HSV transport is similar to

that of rabies virus, diphteric toxin or immunoperoxidase. After microinjection into neurons of s. nigra, HSV behaved as a tracer following the axonal projections of this nucleus to the brain cortex (Bak *et al.*, 1978). Because the finding of intraaxonal particles is extremely rare, it can be assumed that the deproteinized replication unit — the core of the capsid — could be transported as well instead of entire virions.

Nonlethal HSV infection

In nonlethal infection HSV usually reaches the pseudounipolar neurons of the regional sensory ganglia where it persists for lifelong (Baringer, 1975; Stevens, 1975). This was repeatedly confirmed by explantation of minced ganglion fragments or by their cocultivation with indicator cells. The explantation of human sensory and autonomic ganglia gave similar results (Baringer, Swoveland, 1973; Rodda *et al.*, 1974).

The ganglion samples are not infectious at the time of their removal, but they yield virus within 3—6 days in culture. Neurons, in which the latency had been established, begin to produce infectious virus either spontaneously or upon stimulation (see later). When the axon is transsected by neurosurgery, neurons in which the virus had been activated, undergo destruction (McLennan *et al.*, 1980). It seems that establishment of latency does not require productive (lytic) HSV replication (Rajčáni and Čiampor, 1978). Highly virulent strains, which grow well in semipermissive pseudounipolar neurons, are less suitable for the establishment of latency because they induce acute ganglionitis with productive involvement of neurons, their satellites and Schwann cells; then they may spread along the nerve root to the Redlich-Obersteiner transition line infecting susceptible astrocytes which are connecting the Schwann cells. In this way the axonal spread, important for the establishment of non-productive latency, is converted to a combined neural spread (intraaxonal plus endoneural transmission) associated with lytic reproduction of the virus. Nonproductive persistence of HSV DNA in the brain stem (Cabrera *et al.*, 1980) may also occur, as the virus reaches the neurons in brain stem also by the axonal route.

Molecular mechanisms of latency

It was suggested that latency means either a nonproductive survival of HSV DNA in neurons (static latency) or a productive continuous production of trace amounts of virus in very few ganglion cells (Roizman, 1965). The hypothesis of static latency seemed to be confirmed by several observations. First, the viral DNA synthesis and the formation of virus-specific structural antigens in ganglion explants starts in culture usually after a lag phase of several days (Rajčáni *et al.*, 1977). Second, the "silent" genome cannot be eradicated from the ganglion cells, not even by treatment with drugs like acycloguanosin (Field *et al.*, 1979), phosphonoacetic acid or phosphonoformic acid (Svennerholm *et al.*, 1981). Third, in the mouse model, viral DNA has been found by hybridization in situ in both acute and chronic ganglion samples, while viral mRNA sequences were detected in the acute samples

only (Puga *et al.*, 1978). The latter finding seemed to be in accordance with the failure to detect the immediate early and early polypeptides in the majority (92%) of chronic rabbit ganglion samples (Rajčáni and Matis, 1981). Recently, it has been shown that the immediate early polypeptide 175K is frequently present in the nuclei of neurons harbouring the latent HSV (Green *et al.*, 1981). This controversy may be explained by different reactivity of the sera: our serum reacted predominantly with the immediate early polypeptide 110K (Matis and Rajčáni, 1980) as the 175K polypeptide was not formed in detectable amounts in the SIRC cells used for preparation of the immediate early antigen extract.

It is interesting to compare the limited expression of at least a single non-structural polypeptide with the reports on the presence of some mRNA sequences in neurons carrying the "latent" virus. In 46% of the ganglia from 71% guinea pigs infected with HSV-2, mRNA sequences were detected in 0.3—5% of neurons by hybridization in situ (Tenser *et al.*, 1982). In human paravertebral ganglia, the expression of at least a part of the HSV DNA was demonstrated (fragment *Hin* IIIB and several other fragments from the left side of the L segment) in 17 out of 40 autopsy cases (Galloway *et al.*, 1982). The detection of mRNA sequences transcribed from the L segment of HSV DNA is not relevant to the translation of the 175K polypeptide, as this protein is coded by the reiterated repeats of the S segment (Mardsen *et al.*, 1978). In this respect of importance are another non-structural proteins coded by the L segment. Thymidine kinase is present in the ganglia in detectable amounts during the first 2 months after the establishment of latency (Yamamoto *et al.*, 1977). As shown in mice infected with ts mutants, the beta polypeptide thymidine kinase is inevitable for the establishment of latency (Tenser *et al.*, 1981) along with at least one immediate early virus-coded protein (Watson *et al.*, 1980).

Reactivation models

The reactivation of HSV in vivo and in the ganglion explants in vitro implies the persistence of the whole viral DNA and its intermittent expression. No doubt, during latency period viral particles (Baringer and Swoveland, 1974) and structural viral antigens (Rajčáni and Čiampor, 1978) were seen in as well as infectious virus had been recovered from the ganglia (Schwartz *et al.*, 1978) in a part of experimental animals. At least a part of human ganglia examined has shown the presence of both DNA and mRNA sequences (Galloway *et al.*, 1979). These results may be interpreted as intermittent expression of the whole genome in contrast to the long-term limited expression of a part of the genome. The clinical counterpart of this is the well known intermittent shedding of HSV in tears, saliva or vaginal secretions repeatedly found in experimental animals and man. In a relatively low rate HSV may be activated spontaneously (Hill *et al.*, 1980), or this occurs upon stimulation in a relatively higher rate. The best documented experimental model is the mechanical stimulation of the skin inoculation area in mice, the so-called skin triggering (Hill *et al.*, 1978). Inflammation

after UV-irradiation has a similar effect. Thus, changes in physiological state of the skin and an irritation of the nerve endings may "switch on" the productive replication in neurons and create favourable conditions for virus replication in skin or mucous membranes. The latter effect is probably mediated by prostaglandins, especially PGE_2 (Harbour *et al.*, 1978). In rabbits, peripheral triggering had been demonstrated using epinephrine iontophoresis (Known *et al.*, 1981) and xylol-induced keratitis (Kočišová and Rajčáni, in preparation). On the other hand, the latent HSV may be also activated by stereotactic ganglion stimulation, i.e. by ganglion triggering (Nesburn *et al.*, 1976). The effect of transsection of axons in the trigeminal nerve or in other cerebrospinal nerves has been described in patients and experimentally confirmed in animals (Price, Schmitz, 1978). Alternatively, activation of HSV in culture may be explained by interruption of axons upon ganglion removal in addition to the lack of the elimination of the host's immune defence.

Immune response and latency

The participation of immune response in establishment, maintenance and reactivation of HSV latency has become apparent in a series of experiments. When the ganglia from mice with latency were implanted to the peritoneal cavity of passively immunized syngeneic recipients, HSV activation was prevented; in contrast, virus activation occurred in recipients, which had not received the immune IgG (Stevens, Cook, 1974). Alternatively, when immune serum had been added to the explanted ganglion fragments, the activation of HSV was markedly reduced (Rajčáni *et al.*, 1977). The immunocompetent T lymphocytes accumulate mainly in the lymph nodes draining the inoculation site (Morahan *et al.*, 1977). The addition of such cells to the explants in vitro may reduce the activation rate at least 4 times (Rajčáni, Nash, unpublished). The extent of latency, i.e. the number of neurons which become virus carriers can be reduced by passive (Openshaw *et al.*, 1979) or active immunizations (Rajčáni *et al.*, 1980; Klein *et al.*, 1981). Systemic immunity does not prevent local replication of HSV in the cornea, but confers to nerve endings a certain degree of protection against penetration and neural spread of HSV, thereby reducing the number of neurons affected by the virus. Local accumulation of lymphocytes in the regional lymph node seems to be essential, because infected mice treated with phosphonoacetic acid and showing low or undetectable levels of antibodies can be reinfected at a distant site, but they are resistant to reinoculation at the site of primary infection (Klein *et al.*, 1978). The rate of spontaneous HSV activation can be enhanced by the immunosuppressive drug cyclophosphamide (Hough, Robinson, 1975; Kurata *et al.*, 1978). The latter authors also showed the relationship between the fluorescence of viral antigens in neurons and reappearance of the virus at the inoculation site, which is usually free of virus during latency period. The reversed movement of activated virus ("round trip") had been confirmed in many animal models and was widely accepted by the majority of investigators (reviewed by Klein, 1982). An exception in

this respect is the guinea pig model (Scriba, Tatzber, 1981), showing extraneural productive persistence of HSV in tissues surrounding the inoculation site.

While the role of immunocompetent T_k lymphocytes, NK cells and complement mediated cytolysis in acutely infected target cells is well documented, the exact mechanism of the immune control maintaining the HSV latency is not quite clear (reviewed by Babiuk, Rose, 1979). Lehner's (1975) hypothesis suggested binding of IgG by one end to virus-induced Fc receptor and by its antigen-reactive site to virus-coded antigen(s). This positive control seems to be attractive in the light of early expression of certain non-structural virus coded protein(s) which may occur also in the course of nonproductive virus-neuron interaction. Cells infected with bovine herpesvirus express on their surface a non-structural early glycoprotein (Misra *et al.*, 1982). In addition, several other herpesviruses (i.e. EB virus, Marek's disease virus) express both early as well as late virus-coded surface antigens and especially the former seem to be important in the initiation of immune signalling. On the other hand neurons carrying the "silent" genome escape immune destruction, which occurs in association with activation of the latent virus. The negative immune control as suggested here, means the elimination of few neurons producing infectious virus in the absence of any signs of clinical disease (i.e. during inapparent virus shedding). Indeed, in recurrent herpetic disease impaired lymphocyte responses to HSV have been demonstrated (Lopez, O'Reilly, 1977; Kirchner *et al.*, 1978*b*). The negative control would also fail in immunosuppression or upon ganglion stimulation (central triggering) when the number of neurons reproducing the activated virus is relatively high. The immune destruction of virus-producing neurons in the ganglion would not prevent clinical exacerbation in situations when microamounts of virus transported to the UV-irradiated or injured periphery could find favourable conditions for their growth. Thus, reproduction of the virus takes over due to ganglion or skin triggering or in recurent disease because of the enhanced threshold for starting the cell mediated immune response.

Dual behaviour of neurons: an unsolved problem

The either permissive or nonpermissive behaviour of HSV-infected neurons is poorly understood. The complete or incomplete set of HSV genes resides in neurons for lifelong, but it is unclear whether the viral DNA becomes integrated, remains episomal or both. There is no agreement in the question whether the HSV DNA sequences are continuously transcribed (at least a part of them) or whether their expression is intermittent with "silent" periods of static latency. Up to now, there is no direct evidence of a special transcription control for HSV DNA in addition to the generally accepted cascade regulation control (alpha, beta and gamma genes), described in highly permissive cells. It is tempting to imagine that a cellular repressor operating in neurons could reversibly block the expression of the immediate early polypeptide essential for transcription of β-genes. In any case, some

indirect evidence has accumulated suggesting that binding of antibody to cellular surface and the destruction of virus-producing cells are not the only mechanisms of latency control. The search for the link between the antibody-mediated blocking of the virus coded surface antigen and the expression of persisting viral genes would be an interesting topic of investigation in future.

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