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IMMEDIATE EARLY AND EARLY POLYPEPTIDES IN HERPESVIRUS LATENCY

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Summary. — A serum reacting with the immediate early polypeptides (175K, 136K, 110K, 87K, 63K), with at least three early (beta) polypeptides (146K, 44K, 32K) and with the capsid polypeptide 155K of herpes simplex virus (HSV), was used to stain sections from trigeminal ganglia of rabbits with established latent HSV infection. Alternating sections were stained with a serum to partially purified HSV particles. The results of the anticomplementary immunofluorescence by the first serum were compared with the results of the indirect immunofluorescence staining by the second serum in serial sections from both non-cultured and cultured halves of the same ganglion. Nonstructural polypeptides were detected in ganglion cells before culturing only in 1 out of 12 rabbits. A good correlation was found between the occurrence of nonstructural and structural antigens in the explanted fragments starting from 72 hr in culture. The findings were consistent with the hypothesis of static latency provided that spontaneous activation of the silent genome would occasionally occur in a few ganglion cells.

Key words: herpes simplex virus type 1; latent infection; nonstructural polypeptides; immunofluorescence

Introduction

Over the last decade evidence has accumulated in favour of the hypothesis of static latency (reviewed by Stevens, 1978). Puga *et al.* (1978) detected virus-specific DNA sequences in both acute and chronic stages of latency in the corresponding sensory ganglion samples; virus-specific mRNA molecules were found only in the acute stage, but not in the course of latency itself.

The gradual decrease in the amount of virus-coded thymidine kinase was reported by Yamamoto *et al.* (1977). During the first 4 weeks after infection, the levels of the enzyme decreased below the threshold of detec-

tibility. A quantitative analysis of the number of neurons containing structural HSV antigens in serial sections of rabbit trigeminal ganglia showed 1 positive out of 500 neurons in 4 of the 6 samples examined. By contrast, during the stage of latency, 1 positive neuron was seen among from 2000 to 5000 negative neurons in 3 out of 7 rabbits (Rajčáni and Čiampor, 1978). The latter finding is in good agreement with the report by Baringer and Swoveland (1974), who found viral particles in very few neurons of the sensory ganglia from 3 out of 9 rabbits examined in the chronic stage. In addition, virus-specific mRNA sequences were detected by hybridization in situ in neurons of some human sensory ganglia (Galloway *et al.*, 1979). The direct isolation of infectious HSV from a portion of the sensory ganglia during latency was reported following the use of an extremely sensitive detection system (Schwartz *et al.*, 1978). Thus, in the course of nonproductive persistence of HSV DNA in ganglion cells, an occasional switch to the expression of the genome seems to occur; or, alternatively, the degree of its expression rises above the level of the sensitivity of the currently used methods.

In our work, serial sections from the ganglion and its explanted fragments were stained for immediate early and early (nonstructural) and structural HSV antigens.

Materials and Methods

Rabbits were inoculated into the right scarified cornea with 10^6 plaque forming units (PFU) of the Kupka strain of HSV 1. After 6—7 months, both trigeminal ganglia, trigeminal roots and their entrance zones into the brain stem were removed. The ganglia were halved and either immediatelly frozen or minced into small fragments. The latter were cultured in CMRL-1415 medium as described (Rajčáni *et al.*, 1977). The other tissues listed above were similarly minced and kept for 7 days in the same medium containing 10% foetal calf serum. The medium was changed on day 4. At intervals starting from 48 hr in culture, the ganglion fragments were removed. Explants originating from the same ganglion were collected, quickly frozen and cut in a cryostat. Serial sections from the ganglia and their cultured fragments were alternatively stained by immunofluorescence (IF) with two different sera. The serum designated anti-IEE reacted with the polypeptides synthesized during the first 4 hr after the removal of the cycloheximide block (Matis and Rajčáni, 1980). The following polypeptides were identified in the immunoprecipitates of infected SIRC cells with this serum in polyacrylamide gel electrophoresis: (*1*) immediate early (alpha) proteins 175K, 136K (probably identical with 0 polypeptide described by Pereira *et al.*, 1977), 110K, 87K and 63K; (*2*) early (beta) polypeptides 146K, 44K and 32K; and (*3*) the major capsid polypeptide 155K. The second serum was prepared against partially purified HSV particles and reacted with the structural virion polypeptides. Both sera raised in rabbits. Sections treated with the anti-IEE serum were washed, incubated with commercial guinea pig complement, washed in saline and stained with a conjugate to guinea pig C3 component (Dynatech, Switzerland). After washing, the sections were treated with anti-rabbit Ig (Sevac, Prague) conjugate. This modification of the anticomplementary immunofluorescence (ACIF) technique yielded bright specific fluorescence and will be referred to as the combined ACIF technique. Sections treated with the anti-HSV serum were stained only with the anti-rabbit-Ig conjugate (indirect IF method).

Results and Discussion

The results are summarized in Table 1. There was a good correlation between the number of positive ganglion samples as revealed by the two sera used. The incidence of positive homolateral ganglion samples approximated

Table 1. Detection of structural and nonstructural HSV antigens in the homolateral trigeminal ganglion in the course of experimental latent infection of 12 rabbits

Type of serum	Not cultured*	Ganglion Days in culture** 2	3	4	6
anti-IEE	1/12	0/6	2/10	3/10	10/12
anti-HSV	1/12	0/6	2/10	2/10	8/12

* One half of the homolateral trigeminal ganglion was quickly frozen, cut in serial sections which were alternatively stained with either serum; positive ganglion cells seen in 1 rabbit (8.3%).

** The second half the same ganglion was kept in culture. Positive fluorescence occurred in 83% of ganglion samples.

the total number of those examined between 4—6 days in culture. Single neurons showing the presence of both nonstructural and structural HSV antigens were seen before culturing only in 1 ganglion sample. Ganglion explants positive with the anti-HSV serum were also positive when stained for IEE antigens in parallel sections. On the other hand, not all ganglion samples stained with the anti-IEE serum were positive in parallel sections stained with the anti-HSV serum. In 2 out of 12 contralateral trigeminal ganglia, positive staining by either serum was seen on day 6 in culture.

In some ganglia only a few neurons showed the presence of IEE antigens in their nuclei and cytoplasm (Fig. 1). In other explants the positive neurons were more numerous. Satellite cells revealing IEE antigens in their nuclei and cytoplasm were found surrounding positive neurons (Fig. 2), but they could be also seen in the vicinity of negative neurons (Fig. 3). Sections revealing brilliant fluorescence of many neurons and nonneural cells were occasionally seen in explants, which were positive with both sera; such samples revealed positive staining of single neurons already 48 hr earlier (Fig. 4). In these explants, secondary infection of the previously negative ganglion cells cannot be excluded. Sections, in which fluorescence with the anti-IEE serum was more abundant, showed a fluorescence pattern similar to that of the anti-HSV serum. This might be explained by induction of Fc receptors in the later stage of HSV replication in neurons, which then bind the Fc portion of any immunoglobulin. As previously described (Matis and Rajčáni, 1980), the anti-IEE serum stained predominantly the intranuclear antigens, when applied to infected cells between 3—6 hr p.i. Neurons showing nuclear fluorescence were clearly identified in the sections (Fig. 2).

As noted above, the majority of the results published so far directly or indirectly support the idea that the HSV genome persists in neurons of the sensory ganglia in a "silent" or "covert" state. In man, sensory ganglia

may contain defective HSV genomes, which can be rescued by means of ts mutants (Brown *et al.*, 1979). This seems to be in accordance with the present results, in that positive staining of the IEE antigens occurred after an interval of at least 3 days in culture (lag phase). The occasional findings of viral mRNA, viral antigens and virus particles in the neurons during the chronic stage are not necessarily contradictory to this notion.

Summing up, two interpretations may be offered. The first would imply an increase of virus-producing ganglion cells above the threshold of their detectability by current techniques (probably at least 2—4 positive neurons have to be present to find some of them in the serial sections of the ganglion; alternatively about 400 mRNA molecules must be present per ganglion to detect them). The second explanation means that the "silent" genome may, but need not, undergo expression. The switch to productive replication is governed by both cellular (Sesikawa *et al.*, 1980) and immune (Openshaw *et al.*, 1979) mechanisms. Whether the productive replication of HSV in single neurons becomes the source of repeated peripheral shedding or induces a recurrent lesion depends of the immune status (Kurata *et al.*, 1978) and of the extent of virus activation in the ganglion. The latter may be influenced by ganglion stimulation (Nesburn *et al.*, 1976) or by peripheral stimuli (Hill and Blyth, 1976).

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Explanation of Micrographs (Plate XLVIII):

Fig. 1. Positive fluorescence of IEE antigens in a few neurons in 1 out of 16 semiserial sections of the contralateral trigeminal ganglion kept in culture for 6 days. × 90, combined ACIF method.

Fig. 2. Positive fluorescence of IEE antigens in the nucleus of a neuron (nucleolus negative) and in the nuclei and cytoplasm of satellite cells surrounding a positive and a negative neuron. Day 6 in culture, combined ACIF method, × 600.

Fig. 3. Positive fluorescence in the nucleus and cytoplasm of a satellite cell. Day 6 in culture, combined ACIF method, × 600.

Fig. 4. Larger group of positive neurons and satellite cells in 3 out of 26 semiserial sections. Day 6 in culture, combined ACIF staining, × 200.