Inhibition of the in vitro-Reactivation of Latent Herpes Simplex Virus Infection in Spinal Ganglia: Comparison of Various Immune Factors

Brief Report

By

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With 1 Figure

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Summary

In order to study whether the latency of herpes simplex virus (HSV) is immunologically controlled, the influence of different immune mechanisms on the in vitro-reactivation of the virus in latently infected lumbosacral ganglia of mice was investigated.

Combined addition of macrophages and antibodies to cultures of ganglionic tissue proved most effective in delaying virus reactivation. This was achieved to a lesser degree when applying antibodies only, whereas macrophages alone were not effective, nor were immune lymphocytes, nor was interferon from L-cells or from the peritoneal cavity of mice.

Research on the factors inducing an acute productive infection of herpes simplex virus (HSV) in the spinal ganglia to convert into a latent persisting infection is still in an early phase. Equally unknown are the mechanisms responsible for maintaining a latent state of infection. The question whether specific antibodies play a decisive role is still controversial, while the influences of cell-mediated immunity and unspecific defense mechanisms as well as the inherent non-permissivity of neuronal cells are also being discussed (6, 7, 8, 10, 11). Stevens and Cook (11) achieved a reduced virus reactivation by implanting the ganglia, enclosed within a cell-tight capsule, into the peritoneal cavity of immunized mice, concluding that antibodies suppress viral reactivation. More recently, Rajcani et al. (8) came to similar
results in experiments applying antibodies to in vitro-cultures of latently infected ganglionic tissues. Results from other in vivo-experiments, however, did not support the presumed role of antibodies: Latency established in passively immunized mice, was sustained, although the animals lacked antibody production (10). This prompted us to compare the influences of various immune factors on the in vitro-reactivation of latent HSV.

Four to six week old C57 Bl mice were vaginally infected with \(10^6\) ID\(_{50}\) HSV-1, and a vaginal swab was taken three days later. The lumbosacral ganglia L3—S4 of mice with positive swab cultures were explanted after at least four weeks post infection (p.i.) as previously described (9). The excised ganglia of each side were collected separately, minced, divided in two equal parts, and given to two 25 cm\(^2\) tissue culture flasks of human embryo lung fibroblasts (HEL). Two of these cultures — one from each side — served as test cultures for the different test reagents, the remaining two served as controls. The cultures were examined microscopically every following day. Virus reactivation in cultures displaying a cytopathic effect (CPE) was ascertained by a subculture of the supernatant on Vero-cells. (This procedure was omitted in the case of the antibody-containing cultures). The incidence of virus reactivation was concluded from the results 30—32 days after explantation, the delay of reactivation was represented by the reactivation rate 12 days after explantation, since virus reactivation became apparent in nearly all of the control cultures up to this time.

Peritoneal macrophages from syngeneic mice were obtained from the peritoneal cavity after stimulation with 1.5 ml thioglycolate broth on three consecutive days. \(5 \times 10^6\) cells were seeded on 25 cm\(^2\) tissue culture flasks. In order to eliminate fibroblasts, the cultures were trypsinized on the second day of culture and subsequently HEL-cells were added. When a considerable decline of macrophages was observed, new cells were added to the cultures.

Spleen lymphocytes were obtained from mice vaginally infected with HSV-1 and boosted by i.p. injection of \(4 \times 10^6\) ID\(_{50}\) of HSV-1 four to six days before extirpation of the spleen. The lymphocytes were prepared by the standard method (5), and \(8 \times 10^6\) cells were seeded on 25 cm\(^2\) tissue culture flasks. Fresh lymphocytes were added every three days.

None of the immune factors listed in Table 1 inhibited the virus reactivation significantly for the complete observation period. A significant delay of reactivation, however, was achieved by antibodies and even more by the combination of antibodies and macrophages, whereas macrophages alone were not effective, nor were immune lymphocytes. The delay of reactivation by antibodies in combination with macrophages is also evident from Fig. 1. By means of endpoint dilutions of active virus it was ascertained that multiplication and spread of active virus were not inhibited in cultures prepared in the same way as the test cultures (not shown). We had presumed the observed effect of antibody supported by macrophages on latently