Herpes Simplex Virus Infection in Human Monocyte Cultures: Dose-dependent Inhibition of Monocyte Differentiation Resulting in Abortive Infection

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SUMMARY

Monocyte-enriched cultures of human blood leukocytes were exposed to herpes simplex virus (HSV) at different multiplicities of infection (m.o.i., from about 10 to 0.0001 p.f.u./cell). Highest maximum progeny virus titres were invariably obtained with low initial m.o.i., i.e. those between 0.01 and 0.0001 p.f.u./cell, while little if any infectious progeny was produced in cultures inoculated with the highest virus concentrations. By the time of maximum virus production, i.e. 5 to 7 days after inoculation, monocytes in the uninfected cultures had mostly differentiated to macrophages. This differentiation was partially inhibited in cultures initially exposed to the higher concentrations of HSV. Synthesis of HSV antigens was detected by indirect immunofluorescence both in the high m.o.i. cultures and in the productively infected cultures. By this criterion, a maximum of 10 to 15% of all adherent cells became infected in both culture types. It is suggested that the higher doses of HSV, by inhibiting cellular maturation, also prevent the subsequent completion of its own infectious cycle.

Mononuclear phagocytes have been shown to have a central role in the pathogenesis of several experimental virus infections in animals. Genetic resistance of mice to certain virus infections (Bang & Warwick, 1960; Goodman & Koprowski, 1962; Lindenmann et al., 1978), as well as the post-natal maturation (Johnson, 1964; Zisman et al., 1970) of the in vivo host defences towards experimental intraperitoneal virus infections, coincide with a similar in vitro resistance or maturation of peritoneal macrophages from the respective inbred strains of mouse (Mogensen, 1979). Spreading of an intraperitoneal herpes simplex virus (HSV) infection in mouse to the liver (Mogensen, 1977) as well as to the central nervous system (Johnson, 1964) is also determined by the susceptibility of peritoneal macrophages to the infection. Much less information is available about the pathogenetic significance of virus–mononuclear phagocyte interactions in the infections of man. It is known that in generalized HSV infection the virus can harbour and spread throughout the body in the leukocyte fraction of blood (Graig & Nahmias, 1973), but the actual host cell type involved in this phenomenon has not been identified. According to previous reports, neither unstimulated lymphocytes nor freshly isolated monocytes are able to support the growth of HSV in vitro. After mitogenic stimulation, however, both T- and B-lymphocytes seem to be suitable hosts for virus propagation (Kirchner et al., 1977; Kirchner & Schröder, 1979). Human monocytes incubated in culture for a few days transform to macrophages, and according to recent reports, concomitantly acquire the capability to support HSV replication (Daniels et al., 1978; Plaeger-Marshall & Smith, 1978).

In the present experiments cultures of human monocyte-enriched blood leukocytes were prepared as described by Alitalo et al. (1980). More than 90% of the cells were initially phagocytic (Hovi et al., 1977) and/or positive in the non-specific esterase (ANAE) histochemical staining (Horwitz et al., 1977). The percentage of cells showing either or both of these monocyte markers further increased during culture.
Fig. 1. Replication of HSV-1 in human monocyte cultures. One-day-old monocyte-enriched leukocyte cultures were infected with different dilutions of HSV-1 produced in human embryonal skin fibroblasts, titre 2.6 × 10^6 p.f.u./ml. Twenty-five μl of 10-fold dilutions of virus were inoculated in Linbro wells (1.5 ml of growth medium). Approximate initial multiplicities of infection (p.f.u./cell): • •, 0.1; ■—■, 0.01; ○—○, 0.001; □—□, 0.0001. Titres indicated represent total infectious virus. Arrow at symbol means a titre as high as or higher than indicated. The quantification of infectious virus was done by a standard plaque assay technique (Vaheri et al., 1969) using cultures of BSC-1 cells. Cell monolayers on Petri dishes were inoculated with 0.2 ml portions of 10-fold dilutions of the preparation to be tested. Plaques were counted after 4 to 5 days of incubation.

Fresh cultures (<24 h) were inoculated with HSV type 1 (HSV-1) preparations at different multiplicities of infection (between about 10 and 0.0001 p.f.u./cell, 10-fold dilutions of virus in each experiment) and incubated at 37 °C. The stock virus preparations were of HSV-1, Tyler strain, propagated with diluted inocula (m.o.i. about 0.1 p.f.u./cell) in cultures of human embryonal skin fibroblasts. The cultures inoculated with the highest multiplicities (from 10 to 0.1 p.f.u./cell) produced little, if any, infectious progeny virus, while diluted inocula (from about 0.01 to 0.0001 p.f.u./cell) readily replicated in the cultures (Fig. 1). The maximum progeny virus titres were obtained about 5 to 7 days after inoculation.

The monocyte origin of the virus-producing cells was documented by first staining for HSV antigen by indirect immunofluorescence (Leinkiki, 1973) and then for ANAE. Cells showing HSV-specific fluorescence were constantly positive for the ANAE marker.

It could be argued that in the case of productive infection the virus initially did not adsorb to monocytes but remained in the culture medium until sufficient differentiation had taken place. This is unlikely, however, because practically no infectious virus could be detected in these cultures during the first few days of infection. Furthermore, some cells (<1%) containing HSV antigens, as judged by indirect immunofluorescence, were seen in the low m.o.i. cultures even after 24 h of infection, indicating that the infectious cycle was initiated while still at the monocyte stage. The percentage of cells containing virus antigen increased with time, reaching a maximum of 10 to 15 % by the end of the observation period. In the high m.o.i. cultures the maximum percentage of fluorescent cells (10 to 15 %) was seen within the first 1 to 2 days of infection. During the subsequent 4 to 5 days, the proportion of cells containing HSV antigen decreased below 5 %.

It is known that defective interfering (DI) particles can be generated during HSV infection in vitro, especially by successive undiluted passages (e.g. Schröder & Urbaczka, 1978). Our
HSV-1 laboratory strain had been kept in BSC-1 or human skin fibroblasts for a number of passages without any signs of the presence of DI particles. To investigate this possibility in the present virus preparations, 10-fold dilutions of virus, resulting in multiplicities similar to those used in monocyte cultures, were inoculated into cultures of human skin fibroblasts. No high multiplicity interference was seen in these fibroblast cultures. Furthermore, the phenomenon in monocytes was equally demonstrated with a fresh HSV-1 isolate kindly provided by the Diagnostic Laboratory of this Department, propagated for only two passages in cultures of human embryonic skin fibroblasts. These results suggest that DI particles do not play a major role in generation of the high multiplicity interference of HSV replication in monocyte cultures. However, they do not exclude the possibility that DI particles, presumably present in small amounts in the inoculum virus are, for some reason, replicated particularly efficiently in monocytes or alternatively, that replication of HSV is more sensitive to the interfering action of DI particles in monocytes than e.g. in fibroblasts.

The high multiplicity interference could have been due to interferon or some other putative inhibitory soluble contaminant (Morahan et al., 1980) in the crude inoculum virus preparations. To test this possibility we produced partially purified virus preparations by first centrifuging infected culture media at 10,000 g for 10 min and then pelleting the virus from the supernatant by centrifugation at 22,000 rev/min in a Beckman SW27 rotor for 75 min. A similar high multiplicity interference was obtained with these virus preparations as in cultures infected with the crude stock virus. Thus a putative soluble contaminant in the inoculum does not seem to cause this phenomenon. Neither was the phenomenon dependent on the host cell type used to propagate the inoculum virus. High multiplicity interference was constantly observed with all tested stock virus preparations, i.e. HSV-1 preparations produced in adult or embryonic human skin fibroblasts, BS-C-1, Vero or L-132 cells or in monocyte cultures, as well as with the prototype strain of HSV type 2 obtained from the American Type Culture Collection and propagated in our laboratory for two passages in human skin fibroblasts.

Another alternative for the high multiplicity interference could be the inhibition of cellular differentiation observed in the high-dose HSV-infected monocyte cultures. During the experiments, cultures were inspected daily with a light microscope and the morphological differentiation to macrophage-like cells was recorded. The majority of the cells in the uninfected cultures was found to differentiate to macrophages within a 1 week incubation period (Alitalo et al., 1980). HSV-1 infection at higher multiplicities (from 10 to 0.1) inhibited this differentiation in a dose-dependent way, with most of the cells exposed to the highest virus doses remaining small and round (Fig. 2). These cultures also showed a greater tendency for cell detachment than the uninfected ones. In contrast, cultures showing the optimum virus replication were indistinguishable in microscopic morphology from the controls.

It has been reported that human monocytes cultured in vitro for a few days acquire the capability to support HSV replication (Daniels et al., 1978; Plaeger-Marshall & Smith, 1978). In our studies infectious progeny virus was recovered from the productively infected cultures only after 2 to 3 days incubation (Fig. 1) when at least some of the cells already showed evident signs of differentiation.

The presence of virus antigens in the high m.o.i. cultures together with lack of infectious virus progeny can be taken as evidence for an abortive infection, in accordance with the earlier report of Daniels et al. (1978).

These results suggest that exposure of human monocytes to a relatively high dose of HSV results in inhibition of cellular maturation which in turn prevents completion of the infectious cycle of the virus, resulting in abortive infection.

The mechanism of HSV-induced inhibition of monocyte differentiation is not known but one plausible candidate is interferon, presumably induced by HSV infection in monocytes.
Fig. 2. Inhibition of monocyte differentiation by high multiplicity HSV infection. Monocyte cultures (see text and Fig. 1) were infected with different m.o.i. of HSV-1. Photographs were taken after 6 days incubation and May–Grünwald–Giemsa staining. (a) A high m.o.i. culture without infectious virus progeny. Cells are small like fresh monocytes. (b) A productively infected culture inoculated with a multiplicity $10^{-4}$ of that in (a). Cells have differentiated to macrophages. (c) Control culture without virus infection and showing typical macrophages.
This view is supported by the fact that relatively low doses of purified human leukocyte interferon added to monocyte cultures can reversibly induce an inhibition of monocyte maturation morphologically similar to that described in the present paper (Lee & Epstein, 1980; T. Hovi et al., unpublished results). Studies on the possible induction of interferon by HSV infection in monocyte cultures are in progress.

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REFERENCES


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