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SUPPRESSION OF MATRIX PROTEIN SYNTHESIS BY HERPES SIMPLEX VIRUS IN BOVINE SMOOTH MUSCLE CELLS

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Summary: We investigated the effect of herpes simplex virus type 1 infection on the synthesis of extracellular matrix proteins by bovine smooth muscle cells. The cells supported viral replication, which reached maximum at 24 hrs post-infection. Uninfected and infected (multiplicity of infection of 5 or 20) cultures of smooth muscle cells were labeled with [^{14}C]proline at five hrs post-infection. Incorporation of radioactivity into non-dializable protein was determined following electrophoresis of the cell-matrix and medium fractions. The synthesis of fibronectin and collagen types I and III was almost completely suppressed. The data suggest that HSV-1 infection of smooth muscle cells *in vitro* alters extracellular matrix synthesis.

Introduction: In recent studies, we have demonstrated that HSV-1 and HSV-2 infect bovine and human EC *in vitro* (1, 2). Such infection leads to induction of Fc and C3b receptors as well as increased granulocyte adherence on endothelial cells (3, 4). Another consequence of HSV infection has been the suppresion of matrix protein synthesis in bovine (5) and human EC (6, 7). Since viral infection has been implicated in the pathogenesis of vascular diseases including atherosclerosis (8-10), we decided to extend our studies and examine the effects of HSV-1 infection in bovine SMC. Our studies show that bovine SMC support the growth of HSV-1. Infection with this virus results in the suppression of synthesis of fibronectin and collagen types I

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Abbreviations: HSV-1 and HSV-2, Herpes Simplex Virus Type 1 and Type 2; SMC, smooth muscle cells; MOI, multiplicity of infection; PI, post-infection; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; EC, endothelial cells; PFU, plaque forming unit; CPE, cytopathic effect.

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and III. The suppression of host-cell protein synthesis is virus dose dependent.

Materials and Methods

Cell Culture: Bovine aorta smooth muscle cells (repository number AG2410) was obtained from the Institute for Medical Research, Camden, New Jersey. The cells were propagated, maintained and subcultured in the medium recommended by the provider institute. Cells were grown at 37°C in a humidified chamber supplied with 5% CO_2 in air.

Virus Infection: NS strain of HSV-1 (at the 18th passage) was used essentially as described by Friedman et al. (1). Vero cells and SMC monolayers grown in 24-well tissue culture plates (Costar, Cambridge, Massachusetts) were used to conduct plaque assays for titration of the virus pool and development of HSV-1 multiplication curves. The plaque assay used was a modification of the method of Schmidt et al. (11). The first overlay was composed of (vol/vol) of MS media (Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania) and 1.2% agarose. The number of plaques was counted at the end of the third day PI and recorded for later use. SMC were either mock-infected or infected with HSV-1 at three MOIs, 0.1, 5 and 20. The combined cell plus medium fractions were harvested at appropriate time intervals designated as shown in Figure 1 and titered for virus by the plaque assay.

Light Microscopy: Mock infected and HSV-1 infected monolayers were examined under the light microscopy for cytopathologic changes at different time intervals PI.

Metabolic Labeling: Confluent 25 cm^2 culture flasks (Costar, Cambridge, Massachusetts) of SMC were infected for 1 hour with HSV-1 (MOI of 5 or 20) to study the effect of viral infection on matrix protein synthesis. Control monolayers were simultaneously mock-infected. One hour after inoculation, unadsorbed virus particles were removed, the cultures were rinsed 3x with PBS and then refed with SMC growth medium. At 5 hours PI, the medium was removed and replaced with fresh medium supplemented with 6 µCi/ml [^{14}C]proline (250 mCi/mmol) (7) (Amersham Corporation, Arlington Heights, Illinois). After labeling for 5 hours, the medium and cell-matrix fractions were separated and processed for SDS-PAGE according to Leammli (12) as described by Kefalides and Ziaie (7). Fluorography was carried out according to Bonner and Laskey (13) using En^{3}Hance (New England Nuclear, Boston, Massachusetts). X-O-Mat XAR-5 Kodak films were exposed to dried gels.

Electroimmunoblot: To identify type III procollagen, medium from mock-infected and infected (MOI = 10) cultures of SMC were collected at 24 hours PI. Samples were subjected to SDS-PAGE and type III procollagen was detected by electroimmunoblot as described by Towbin et al. (14) using a monoclonal antibody against human type III collagen (a gift from Dr. Edward J. Macarak of the Connective Tissue Research Institute).

Results

Bovine SMC supported the growth of HSV-1 at all three MOIs tested, i.e. 0.1, 5 and 20. Figure 1 shows growth curves of virus at various times PI. It will be noted that maximum titers occurred at about 24 hours PI irrespective of the MOI used and then followed a slow decline over the next three days.

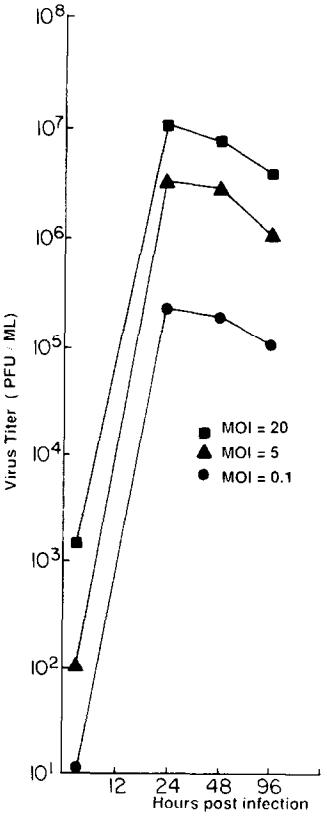

Figure 1. Growth curves of HSV-1 after inoculation onto bovine aorta SMC cultures.

The virus titer at 24 hr PI was proportional to the number of virus particles used in the inoculum reaching over 10^7 PFU/ml with a MOI of 20, 10^6 with a MOI of 5 and 10^5 with a MOI of 0.1.

Further evidence of infection of SMC with HSV-1 was obtained by assessing the degree of CPE. Figure 2A shows a monolayer of uninfected SMC. Figure 2B shows a culture of infected (MOI = 5) SMC 24 hours PI. Dramatic changes, which occurred as early as 3 hours PI (unpublished data), are evident in the appearance of the cells characteristic of HSV-1 infection in vitro. The infected cells appeared rounded and retracted leading to increase in the intercellular space. Note that cells with normal appearance are also present. It should be noted that although rounded, the majority of the cells at this time are still attached to the flask surface. Figure 2C shows CPE of SMC four days following infection (MOI = 20) with HSV-1. The majority of cells were rounded and were detached from the surface of the flask. Viral

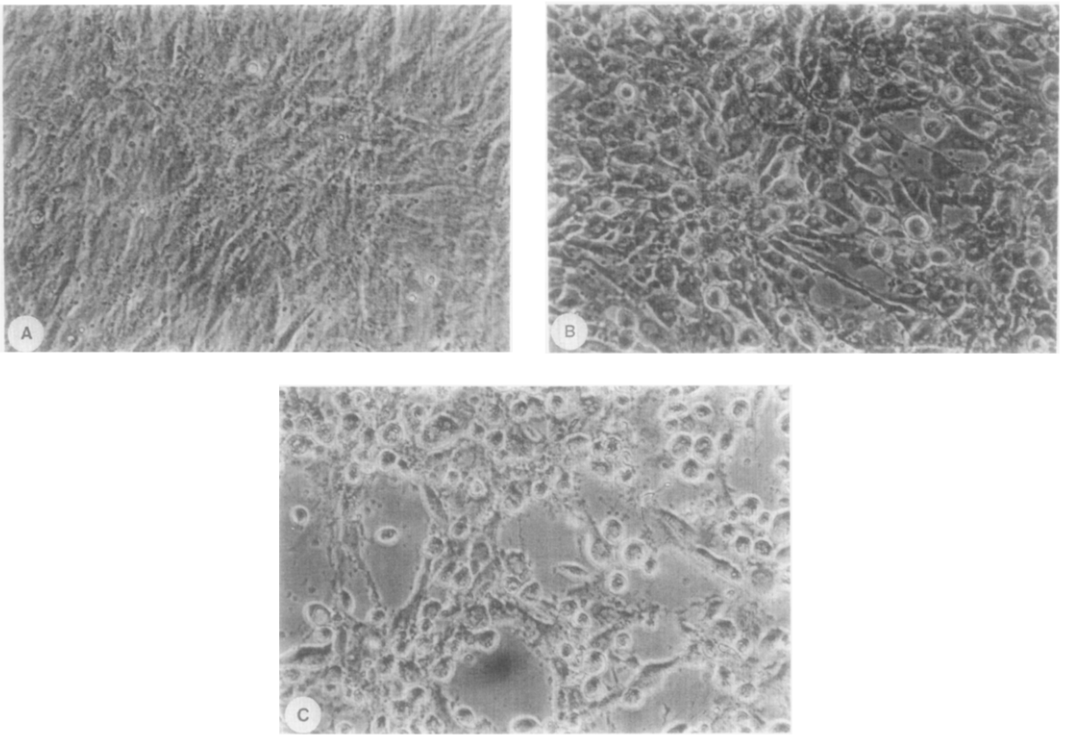

Figure 2. Photomicrograph of monolayers of bovine SMC. A. Mock-infected (25x); B. Infected with HSV-1 at MOI 5. Picture taken at 24 hours PI (25x); C. Infected with HSV-1 at MOI 20. Picture taken at 4 days PI (25x).

antigen in infected SMC was detected by direct immunofluorescence (data not shown).

Matrix protein synthesis was determined in cultures of SMC with and without infection with HSV-1. At 5 hours PI, monolayers were pulsed for 5 hours with [^{14}C]proline. At the end of this period, the medium and cell-matrix fractions were separated and analyzed by SDS-PAGE. Figure 3 shows that HSV-1 infection of SMC suppresses the synthesis of host-cell proteins. It will be noted that labeled proteins corresponding to fibronectin and procollagen types I and III are not detectable in the medium and cell fractions of the infected SMC. Infection with a MOI of 5 appears to be as effective in suppressing matrix protein synthesis as a MOI of 20. In the cell-matrix fraction, we note several labeled bands which correspond to the

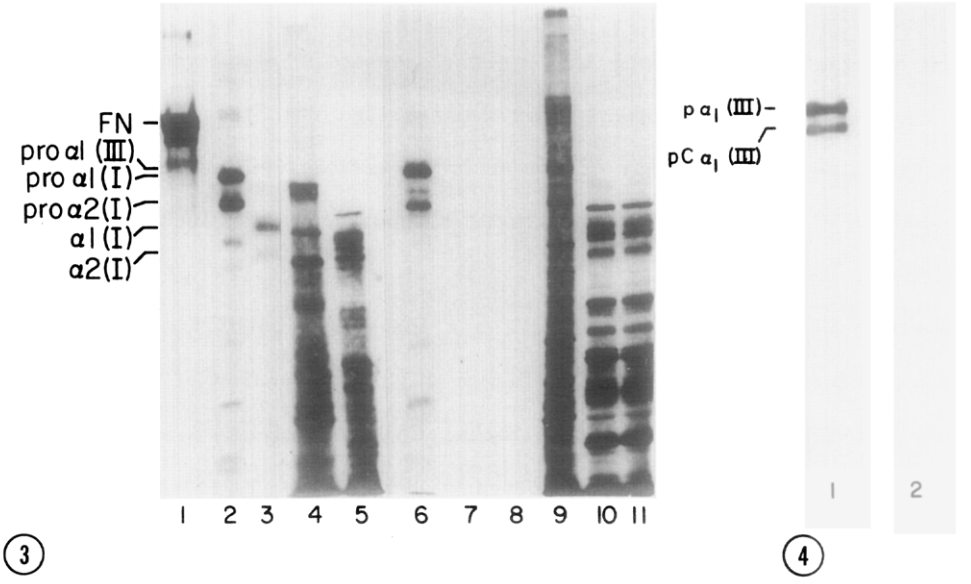

Figure 3. SDS-PAGE pattern of protein synthesis by SMC cultures infected with HSV-1. SMC cultures were either mock-infected or infected with HSV-1 at a MOI Of 5 or 20. At 5 hours PI the cultures were labeled with [^{14}C]proline for 5 hours. The medium and cell-matrix fractions were separated and analyzed by SDS-PAGE. Lane 1, fibronectin (FN) standard; lane 2, pro α1(I) and pro α2(I) collagen chain standards from human foreskin fibroblasts; lane 3, α1(I) and α2(I) collagen chain standards; lanes 4 and 5, and polypeptides of HSV-1, respectively; lane 6, medium from mock-infected cultures; lanes 7 and 8, medium from infected cultures at MOI 5 and 20, respectively; lane 9, cell-matrix fraction from mock-infected cultures; lanes 10 and 11, cell-matrix fraction from infected cultures at a MOI of 5 and 20, respectively.

Figure 4. Electroimmunoblot pattern of the medium fraction from mock-infected (lane 1) and HSV-1 infected (lane 2) SMC cultures, using a monoclonal antibody against type III collagen. α1(III) = pro α1 chain of type III procollagen. pC α1(III) = processed pro α1 chain of type III procollagen still retaining the carboxyl terminus.

newly synthesized polypeptides of HSV-1. It should also be noted that at this time (10 hr PI) no viral polypeptides are detected in the medium.

The synthesis and electrophoretic mobility of type III procollagen was verified by electroimmunoblot using a monoclonal antibody against human type III collagen. It will be noted that there are two reactive bands (Figure 4, lane 1) corresponding to pro α1(III) and pC α1(III) chains of type III procollagen in the medium from uninfected SMC. In the medium from infected SMC no reactive bands are noted (Figure 4, lane 2).

Discussion

The studies presented here demonstrate that bovine aorta SMC *in vitro* support the growth and replication of HSV-1. We have tested SMC as part of

our studies designed to understand the early responses of blood vessel cells to viral injury. In previous studies, we demonstrated that human and bovine EC support the growth of a variety of common human viruses (1-2). As with the human and bovine EC, bovine SMC demonstrated cytopathology characteristic of HSV-1 infection, eventually leading to lysis of the majority of infected cells. However, SMC appeared to persist for a longer period of time (4 days PI) even when infected at a MOI of 20 (Figure 2C). Suppression of host-cell matrix proteins, FN and type III procollagen, occurred early, i.e. between 5-10 hours PI. Similar results were reported by Macarak et al. (5) for bovine aorta EC and by Ziaie et al. (6) and Kefalides and Ziaie (7) for human EC infected with HSV-1 and HSV-2. The latter study (7) also demonstrated that the early suppression of type IV collagen and FN in human EC was not dependent on the synthesis of new viral protein. Earlier studies with HSV-1 infection in Vero cells by Fenwick and Walker (15) showed that infection with HSV-1 leads to disruption of host-protein synthesis as well as inhibition of RNA and DNA synthesis (16). More recent studies (17) show that in Vero cells infected with HSV-1, the early phase of the shut-off involves suppression of synthesis through inactivation of host-cell protein mRNA. The importance of our observations may be related to the ability of infected cells to synthesize and deposit an altered extracellular matrix which contains, in addition to host-cell proteins, viral polypeptides. Unpublished data from our laboratory show that in both EC and SMC infected with HSV-1, viral polypeptides are incorporated into the extracellular matrix. Contrary to this, there is little or no evidence of labeled HSV-1 polypeptides in the medium fraction of infected SMC cultures. It is possible, therefore, that viral infection of blood vessel cells in vivo may lead to an altered extracellular matrix which in turn may influence cellular phenotypic expression.

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