Suppression of Fibronectin Synthesis by an Early Function(s) of Human Cytomegalovirus

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SUMMARY

Labelling of fibronectin with [3H]leucine and its isolation by immunoprecipitation followed by electrophoresis on gels showed that fibronectin synthesis was specifically suppressed in human embryonic cells infected with human cytomegalovirus. Degradation or release of pre-existing fibronectin into the medium was not affected. The inhibition of fibronectin synthesis was also observed when cells were infected with a DNA-minus temperature-sensitive mutant at the non-permissive temperature but not observed in infection with u.v.-irradiated virus, suggesting the involvement of the expression of an early virus function(s). This capacity of the virus may be implicated in the virus-induced early cell rounding.

Fibronectin is a major glycoprotein polymer with subunit mol. wt. of 200,000 to 250,000 (200K to 250K) which is present on the surface of various types of cells and which is at least partially responsible for cell morphology. It is of interest that the amount of fibronectin is reduced in transformed cells and the reduction generally correlates with tumourigenicity of transformed cell lines or propensity of cells to metastasize. This decrease in fibronectin has been ascribed to reduced synthesis, faulty cellular retention, increased degradation, or combinations of these events (for review, see Yamada & Olden, 1978).

Human cytomegalovirus (HCMV), for which potential oncogenicity has long been suspected, induces cell rounding (Furukawa et al., 1973), as well as the activation of cell chromatin (Kamata et al., 1978, 1979) and the synthesis of cellular DNA and RNA in human fibroblasts at early stages of infection (St. Jeor et al., 1974; Tanaka et al., 1978). Cell rounding is induced by an early virus gene function (Hirai et al., 1977; Ihara et al., 1978 b), clearly differentiating cell rounding from the general cytopathic effect of the virus which is manifested after virus DNA replication. Considering the possibility that HCMV induces cell rounding by affecting cell surface fibronectin, we explored changes in the metabolism of fibronectin in HCMV-infected human embryonic lung (HEL) cells.

To determine the rate of fibronectin synthesis, we labelled cells with [3H]leucine starting at various times post-infection and measured the radioactivity incorporated into fibronectin after its isolation from the cells and the culture media by immunoprecipitation and gel electrophoresis (Fig. 1, upper panels). The incorporation of [3H] into cell-associated fibronectin was linear for up to 60 min in either HCMV-infected or mock-infected cells, whereas the labelled fibronectin first appeared in the medium between 20 and 40 min and then accumulated at an increasing or constant rate. This lag represents the time required for transport of newly synthesized fibronectin and its release into the medium (Choi & Hynes, 1979). The rate of fibronectin synthesis was gradually depressed by HCMV infection beginning at 6 h post-infection. The decrease in the release of labelled fibronectin in HCMV-infected culture was roughly proportional to the decrease in cellular synthesis of fibronectin.

The determination of [3H]leucine incorporated into total acid-insoluble material showed that protein synthesis was not generally inhibited by the infection, or it was even stimulated (by about 20%) later than 24 h post-infection, due at least partly to the synthesis of virus
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Fig. 1. Rate of fibronectin synthesis in HCMV-infected cells. HEL cells grown to confluency in 30-mm plastic dishes were mock-infected or infected with HCMV at a multiplicity of infection of 5 and cultured in 2 ml of the medium containing 2% calf serum as described by Kamata et al. (1978). The cells were labelled with 50 μCi/ml [3H]leucine (171 Ci/mmol, New England Nuclear) in a medium containing one-tenth the normal concentration of leucine and 2% calf serum which had been dialysed against Earle's solution. At the times indicated, labelled cells in dishes were washed with phosphate-buffered saline then lysed in 1 ml 50 mM-CAPS buffer (cyclohexylaminopropane sulphonic acid, Sigma) pH 11 containing 1% Triton X-100 according to the method of Olden & Yamada (1977). Insoluble material was removed by centrifugation. Fibronectin extractable with the pH 11 buffer was regarded as 'cell-associated'. Fibronectin was immunoprecipitated from the pH 11 extracts or culture media essentially according to the method of Parry et al. (1979) with anti-fibronectin serum raised in rabbits by injection of human cold-insoluble globulin (CIG) purified as described by Engvall & Ruoslahti (1977). As controls, the same amount of samples were treated with preimmune rabbit serum. Immunoprecipitates were dissolved in 0.1 M tris–HCl pH 6.8 containing 2% SDS, 5% 2-mercaptoethanol and 10% glycerol, by heating at 100 °C for 3 min. Electrophoresis was run on a 5% (w/v) polyacrylamide gel with a 4% stacking gel and in the buffer with SDS described by Laemmli (1970). Human CIG and bromophenol blue were included as markers. After the run, slab gels were stained with Coomassie Brilliant Blue. The bands corresponding to CIG and the area 1 cm above were cut from the gel, dissolved in H2O2, and radioactivity was determined by scintillation spectrophotometry. As controls, immunoprecipitates formed with preimmune serum were also electrophoresed and the radioactivity in the fibronectin area was determined. Control values were in the range of 1 to 5% of those obtained for the immunoprecipitates by anti-fibronectin and these values were subtracted. Times post-infection were: (a) 2.5 h; (b) 6 h; (c) 12 h; (d) 24 h. Upper panels: 3H incorporated into fibronectin in cpm/30 mm dish. Lower panels: 3H incorporated into acid-insoluble material of the cell or the medium (Fig. 1, lower panels).

proteins. To show the specific inhibition of fibronectin synthesis, the amount of 3H incorporated into fibronectin was expressed as the percentage of the amount of 3H in total acid-insoluble material of the cell or the medium (Fig. 1, lower panels).
To examine whether the expression of virus gene functions is required for the suppression of fibronectin synthesis, we used u.v.-irradiated HCMV (at $73.1 \times 10^{-7} \text{J/mm}^2/\text{s}$ for 5 min, infectivity reduction $10^{-2}$). This dose of u.v. light impairs, by more than 90%, the virus function that causes early cell-rounding, but it does not appreciably affect the penetration of virus genomes into nuclei (Hirai et al., 1977). In this experiment, mock-infected cells and cells infected with u.v.-irradiated or unirradiated HCMV were labelled with $10 \mu \text{Ci/ml} [3\text{H}]\text{leucine for } 24 \text{ h starting at 0, 24 and 48 h post-infection, and the radioactivities incorporated into fibronectin were determined. Under these conditions, the HCMV-mediated inhibition of fibronectin synthesis (cell-associated plus released) was marked later than 24 h post-infection; inhibition was by 82% and 89% for 24 to 48 h and 48 to 72 h labelling respectively. However, no significant inhibition was observed in cells infected with u.v.-irradiated virus. An experiment of the same design was also conducted with an early temperature-sensitive mutant, ts589. This mutant is unable to induce virus DNA synthesis and nuclear inclusions but it is able to induce cell rounding and all other early events tested at the non-permissive temperature (Ihara et al., 1978a, b). The infection with ts589 was found to suppress fibronectin synthesis at either the permissive or non-permissive temperature. The degrees of inhibition by the mutant were by 88% and 89% at 34 °C, and 85% and 80% at 39 °C for 24 to 48 h and 48 to 72 h labelling respectively. Moreover, no significant differences were observed in the ratios of $3\text{H}$-labelled fibronectin released/cell-associated between ts589 and wild-type strain infections. This result, together with that of u.v.-irradiated virus, indicates that the expression of an early virus function(s) is required for the inhibition of fibronectin synthesis.

To compare molecular sizes of fibronectin synthesized in HCMV-infected and mock-infected cells, we labelled cells with $10 \mu \text{Ci/ml} [14\text{C}]\text{leucine or [14C]glucosamine for 30 min and 24 h starting at 24 h post-infection, and fibronectin immunoprecipitated was subjected to gel electrophoresis and autoradiography. Cell-associated fibronectin obtained from either the short-term labelled or long-term labelled cells formed two major bands in the fibronectin region, although the later sample gave broader bands (not shown). Such electrophoretic polydispersity of fibronectin is consistent with the previous observation by Crouch et al. (1978) and Choi & Hynes (1979). The bands detected by $[14\text{C}]\text{leucine labelling coincided with those detected by [14C]glucosamine. Fibronectin released into the medium during a labelling period of 24 h was more heterogeneous than that extracted from the cells. In all these experiments, essentially no qualitative difference was observed between fibronectin molecules from mock-infected and HCMV-infected cultures.

To explore whether HCMV infection affects the processes of degradation or release of fibronectin, we infected cells which had been prelabelled with $[3\text{H}]\text{leucine and then followed the fate of prelabelled fibronectin for 36 h. As seen in Fig. 2, cell-associated fibronectin decreased in both mock-infected and HCMV-infected cultures at a similar rate following first order kinetics. In inverse proportion, labelled fibronectin accumulated in the culture medium. The sum of the labelled fibronectin in the cells and the medium was practically constant within the limit of experimental error, suggesting little degradation of fibronectin in both cultures. It seems, therefore, that the HCMV-induced decrease in the release of newly synthesized fibronectin (Fig. 1) is due to a decrease in fibronectin synthesis rather than an alteration of releasing processes or stimulation of degradation. Immunofluorescent staining of mock-infected cells with the fluorescein-conjugated anti-fibronectin Fab' revealed pericellular fibrils of fibronectin which formed a fine continuous meshwork bridging adjacent cells (not shown). The features of the fibronectin meshwork were similar to those reported for other lines of cells (for review, see Yamada & Olden, 1978). When the cells were infected with HCMV, deformation of the fine meshwork of fibronectin became detectable by 6 h post-infection. This was the time when cell rounding...
Fig. 2. Fate of pre-existing fibronectin after HCMV infection. Cells which had been labelled with 10 μCi/ml [3H]leucine for 48 h and washed were mock-infected or HCMV-infected and cultured in medium supplemented with unlabelled leucine. At the times indicated, 3H in cell-associated fibronectin and that released into medium was determined and expressed in ct/min/30 mm dish. ○, Mock-infected, released into medium; □, mock-infected, cell-associated; △, HCMV-infected, released into medium; ▲, HCMV-infected, cell-associated.

also began. Deformation of the meshwork proceeded with time, and at or later than 12 h post-infection, fibronectin fibres were seen entangled, forming thicker web-like structures which were associated with partially clustered cells (not shown). This observation suggested close correlation between cell rounding and deformation of the fibronectin meshwork. We do not know, however, whether such deformation was caused by a decreased supply of newly synthesized fibronectin in infected cells. Although the deformation of fibrils and cell rounding seemed to precede the maximum inhibition of fibronectin synthesis, the above possibility is not improbable because it has been known that the fibronectin meshwork is continuously replenished (Vaheri & Ruoslahti, 1975).

Fibronectin seems to be exceptional among cellular macromolecules in that its synthesis is suppressed at early stages of HCMV infection. The synthesis of other macromolecules so far tested (DNA, RNA and proteins) are generally enhanced (Furukawa et al., 1973; St. Jeor et al., 1974; Tanaka et al., 1978; Hirai et al., 1977) following the activation of cellular chromatin for transcription (Kamata et al., 1978, 1979). Although it is not known whether the capacity of HCMV for suppressing fibronectin synthesis and destroying the fibronectin meshwork is to be correlated with its potential oncogenicity, this possibility deserves consideration.

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REFERENCES


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