Prostaglandins Enhance Intercellular Adhesion of Vero Cells Infected with Herpes Simplex Virus

By D. A. Harbour,* T. J. Hill and W. A. Blyth

Department of Microbiology, The Medical School, Bristol BS8 1TD, U.K.

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SUMMARY

The effect of prostaglandins (PGs) and cyclic nucleotides on adherence between uninfected Vero cells or between uninfected cells and cells infected with wild-type or syncytial strains of herpes simplex virus (HSV) was studied. In the absence of serum, PGE₂ increased adhesion between uninfected cells whereas PGF₂₅ decreased it. However, both PGE₂ and PGF₂₅ increased adhesion between cells infected with HSV and uninfected cells. Moreover, dibutyryl cyclic AMP increased adhesion between uninfected cells and cells infected with HSV, whereas dibutyryl cyclic GMP decreased it.

INTRODUCTION

Herpes simplex virus (HSV) has been found in the eye (Nesburn et al., 1976) and the skin (Scriba, 1977; Hill et al., 1980) of latently infected animals in the absence of clinically apparent lesions but it is not clear what role such virus might play in the development of clinical disease. In their skin trigger theory Hill & Blyth (1976) postulated that changes in local conditions in the skin may determine whether or not a recurrent herpes simplex lesion develops. Such changes in conditions may be produced by various stimuli or triggers which alter the pharmacological status of the skin, for example by increasing the levels of prostaglandins (D. A. Harbour, T. J. Hill & W. A. Blyth, unpublished results).

We have shown previously (Harbour et al., 1978) that prostaglandins E₂ and F₂₅ enhance the spread of HSV in Vero cell cultures, and that, at least in the case of PGE₂, this phenomenon is probably not mediated through the rise in intracellular cyclic AMP induced by treatment with this prostaglandin.

Infection of cells with HSV leads to differences in their 'social behaviour' (Ejercito et al., 1968) probably mediated by alteration of their cell surfaces (Nahmias et al., 1972). One such change is the series of events leading to cell fusion, by which process HSV can spread directly from cell to cell (Hoggan et al., 1960). Fusion of two cells probably requires adhesion of one cell to its neighbour (Poste, 1972) and we have suggested that prostaglandins enhance spread of HSV in vitro by acting on the cell surface (Harbour et al., 1978). Therefore, we have studied the effects of prostaglandins on the adhesiveness of Vero cells both uninfected and infected with HSV.

METHODS

Cell cultures. Vero cells were grown in Medium 199 with 5% foetal calf serum (FCS) as described previously (Hill et al., 1975).

Viruses. The SC16 strain of HSV-1 (Hill et al., 1975) and a syncytial variant (SC16/syn) derived from it by plaque purification were used. The MP syncytial strain (Hoggan & Roizman, 1959) was used in some experiments.

Chemicals. N°,O²'-dibutyryladenosine 3'-5'-cyclic monophosphate (dbcAMP) and N²,O²'-dibutyrylguanosine 3'-5'-cyclic monophosphate (dbcGMP) were purchased from Sigma. Prostaglandins E₂ (PGE₂) and F₂₅ (PGF₂₅) were gifts from Dr J. Pike (Upjohn, Kalamazoo, Mich., U.S.A.).

Adhesion assay

The assay was similar to that described by Walther et al. (1973).

Production of labelled cells. The DNA of Vero cells was labelled with [³H]thymidine (Amersham International). The [³H]thymidine was added to the growth medium at a final activity of 10 μCi/ml. Cells were seeded in this medium at a density of 10⁵/ml in 4 oz medical flat bottles, and were incubated at 37 °C for 24 h.
Production of substrate monolayers. Monolayers of Vero cells were grown in 10 × 1 cm glass tubes, which were seeded with 1 ml growth medium containing $2 \times 10^4$ cells and then rolled horizontally at a temperature of 37 °C at 1.2 rev/min for 2 days. This gave an area of confluent cells of about 12 to 15 cm$^2$, covering the whole circumference of the tube, and containing approximately $3 \times 10^5$ cells; these cultures will be referred to as substrate monolayers. The medium was replaced by 1 ml medium containing the drug under test, or control medium, and the cultures were incubated for a further 24 h. For this time the medium contained 5% FCS or no serum (see below). Infected substrate monolayers received 10 p.f.u. HSV per cell and were then incubated for 5 h with fresh control or test medium before being used in the adhesion assay.

The Assay. Labelled cells were removed from the glass with 0.02% EDTA and deposited by centrifugation at 500 g for 10 min. They were then resuspended in medium with or without the drug under test. All drugs were tested in the presence of 5% serum and without serum. After discarding the medium from treated and control substrate monolayers 1 ml of labelled cell suspension containing approximately $2 \times 10^5$ cells (with or without drug) was added to each monolayer. The tubes were incubated at 37 °C and rotated horizontally at 1.2 rev/min. Two tubes treated with drug and two control tubes were taken immediately and at 10-min intervals for 90 min. The medium was discarded and the substrate monolayers were washed three times with medium without serum. The monolayer and any adherent labelled cells were solubilized in 1 ml m-NH$_2$OH and placed in a plastic scintillation vial. Five ml of Dimilume (Packard) or PCS (Amersham International) scintillant were added, and the samples were counted in a Packard Tri-Carb 3330 scintillation counter. The percentage radioactivity bound to the substrate monolayer was calculated; 100% radioactivity was estimated by counting the activity in 1 ml of labelled cells. On average, each ml of labelled cell suspension contained 60000 ct/min (range 16000 to 230000 in different experiments).

Each treatment was tested in the assay on two to four separate occasions.

RESULTS

In the presence of serum, adherence of labelled cells to uninfected substrate monolayers was markedly higher than in the absence of serum (Fig. 1). When substrate monolayers were treated with PGE$_2$, there was an increase in adherence of labelled cells relative to that on untreated monolayers whereas treatment with PGF$_{2\alpha}$ produced a large decrease in adherence. These effects were not influenced by the presence of serum (Fig. 2).

Infection of substrate monolayers with SC16/syn virus decreased adherence compared with uninfected cells whether or not serum was present (Fig. 3a, b); MP virus gave an almost identical result. However, infection with the wild-type SC16 virus had no effect on adherence (Fig. 3c, d).

In the absence of serum, treatment with either PGE$_2$ or PGF$_{2\alpha}$ of monolayers infected with SC16 or SC16/syn resulted in increased adherence compared to infected but untreated controls (Fig. 3a, c). The addition of serum had a marked effect, strongly reducing the increase observed with PGE$_2$ in cells infected with either SC16 or SC16/syn virus. Serum also reduced the increase obtained with PGF$_{2\alpha}$ on cells infected with SC16/syn, but resulted in an even bigger increase in adherence of SC16-infected cultures treated with PGF$_{2\alpha}$ compared to infected controls.
Prostaglandins, HSV and cell adhesiveness

Fig. 2. Adherence of radiolabelled Vero cells to an uninfected homotypic substrate monolayer: (a) without serum; (b) with 5% FCS. Substrate monolayers were treated with: ▲, 10 μg/ml PGE₂; △, 10 μg/ml PGF₃₂α; ■, 50 μg/ml dbcAMP; □, 10 pg/ml dbcGMP. Change in adhesion: each point represents the cumulative difference in percentage radioactivity adherent to the monolayer between the mean result from test cultures and that of their controls at each time. A positive value indicates that adherence was greater in test cultures than in controls.

Fig. 3. Adherence of radiolabelled Vero cells to infected homotypic substrate monolayers: (a, b) monolayer infected with SC16/syn; (c, d) monolayer infected with SC16; (a, c) without serum; (b, d) with 5% FCS. Monolayers were treated with: ●, nothing; ▲, 10 μg/ml PGE₂; △, 10 μg/ml PGF₃₂α; ■, 50 μg/ml dbcAMP; □, 10 pg/ml dbcGMP. The infected, untreated monolayers (●) were compared to uninfected, untreated monolayers. Treated, infected monolayers were compared to untreated, infected monolayers. For details of change in adhesion, see Fig. 2.
Treatment with PGs also had very different effects on adherence depending on whether or not the substrate monolayers were infected. In uninfected monolayers, PGF$_{2\alpha}$ severely depressed adhesion but in monolayers infected with either SC16 or SC16/syn, virus adhesion was increased in the presence of the drug (Fig. 2, 3). In the absence of serum the small increase in adhesion seen when uninfected monolayers were treated with PGE$_2$ was considerably enhanced in infected monolayers.

With the exception of uninfected monolayers in the absence of serum, treatment with dbcAMP always increased adherence. There was no consistent pattern in the effect of dbcGMP on adherence except that in the absence of serum, adherence to infected monolayers was decreased compared with that of infected monolayers without drug (Fig. 3 a, c).

In separate experiments (Harbour et al., 1978) cultures of Vero cells infected with 20 p.f.u./well of SC16 virus were exposed to PGE$_2$ or PGF$_{2\alpha}$ in concentrations of 0.1 to 10 gg/ml. Although addition of PGs caused enlargement of plaques compared to those in control cultures, no increase was seen in the number of polykaryocytes in the plaques.

**DISCUSSION**

Cellular adhesion is almost certainly a phenomenon associated with the cell surface in which cyclic nucleotides play a role (Weiss, 1973). Prostaglandins produce many of their effects by binding to the cell membrane (Johnson & Ramwell, 1974). With PGs of the E series this usually leads to activation of membrane-bound adenylate cyclase, leading to an increase in the intracellular levels of cyclic AMP. On the other hand, PGF$_{2\alpha}$ activates the soluble enzyme guanylate cyclase, leading to increased levels of cyclic GMP, and has little effect on levels of cyclic AMP (Kuehl, 1974). It has been proposed that the control of many cell functions may be elicited by the opposing actions of cyclic AMP and cyclic GMP (Goldberg et al., 1975).

However, not all actions of PGs are mediated through cyclic nucleotides (Johnson et al., 1975; Lillie, 1974; Rasmussen et al., 1975; Polgar & Taylor, 1977).

The common observation that cultured cells attach better to substrates of glass or plastic in the presence of serum is mirrored here in the increased adhesion between cells in medium containing 5% serum. The presence of serum also dramatically affected the activity of the cyclic nucleotides on cell-to-cell adhesion. With serum, dbcAMP increased adhesion between cells, a result similar to that of Johnson & Pastan (1971) with L929 cells. However, Weiss (1973) working with Erhlich ascites tumour cells reported the opposite result. In our own work, dbcGMP decreased adhesion in the presence of serum but not in its absence. As serum is known to reduce the level of cyclic AMP and increase that of cyclic GMP in cells derived from various species (Rudland et al., 1974) it seems that the effects of serum on adhesion are not due to its effect on cyclic nucleotide levels.

With or without serum, PGF$_{2\alpha}$ substantially decreased adherence between Vero cells whereas PGE$_2$ slightly increased it, an example of those circumstances where PGs of the E and F series have opposing effects. These findings are in agreement with those of Weiss (1973) but not with those of Johnson & Pastan (1971) in the case of PGF$_{2\alpha}$. However, it is likely that differing techniques of measuring cell adhesiveness might measure different things which might explain discrepancies in the results obtained in different laboratories.

We showed previously that treatment of Vero cultures with PGE$_2$ or PGF$_{2\alpha}$ enhanced the spread of HSV from cell to cell. By contrast, treatment with dbcAMP or theophylline (each of which raises intracellular cyclic AMP levels) reduced the spread of virus (Harbour et al., 1978). We (D. A. Harbour, T. J. Hill & W. A. Blyth, unpublished observations) and others (Stanwick et al., 1977) have shown that treatment of cells with dbcGMP enhances the yield of HSV after infection of cells at low multiplicity. The latter authors also showed reduced virus yields from cells treated with theophylline, dbcAMP or papaverine, each of which increases cyclic AMP levels.

In our experiments, 5 h after infection with the syn strain, cell adhesion was reduced although no effect was seen with the wild-type virus. Previously, Batra et al. (1978) demonstrated that 18 to 20 h after infection with HSV at a multiplicity of infection of 100, chick heart cells showed decreased cellular adherence. However, at this late stage and with such a high multiplicity of
infection the cells must have been virtually dead. The decrease in adherence after infection by SC16/syn was surprising since it has been suggested that adhesion between cells is a prerequisite of fusion (Poste, 1972). However, when cells infected with either wild-type or syn virus were treated with PGE₂, PGF₂α or dbcAMP in the absence of serum, the cells were more adhesive than infected, untreated cells or uninfected, treated cells. This was particularly marked in the case of treatment with PGF₂α, which increased adherence of infected cells whereas it had decreased adherence of uninfected cells. The result with PGE₂ is consistent with the suggestion that its action may be through its effect on cyclic AMP. On the other hand PGF₂α is unlikely to be acting through cyclic GMP since cells infected with wild-type virus and treated with dbcGMP were less adherent than uninfected, treated cells or infected, untreated cells. Although PGs did not induce polykaryocyte formation in cultures infected with SC16, the increased adherence caused by PGs might facilitate cell-to-cell spread of virus and explain the previous observation that PGs increase spread of HSV infection through cultures.

Hill (1981) discussed possible mechanisms of induction of recurrent herpes simplex and put forward the possibility that induction might be controlled both in the skin and in the ganglion. Control in the skin may be mediated by PGs as these compounds are induced in the skin by all stimuli so far examined that induce recurrent disease. If PGs enhance the adherence of cells infected with HSV to uninfected cells in vitro, this could make development of recurrent disease more likely in that the spread of virus from 'microfoci of infected cells' (Hill & Blyth, 1976) in the skin might be enhanced.

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REFERENCES


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