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THE RELATIONSHIP BETWEEN THE ANTIVIRAL ACTION OF INTERFERON AND PROSTAGLANDINS IN VIRUS-INFECTED MURINE CELLS

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The relationship between prostaglandins (PG) and interferon (IFN) was investigated. IFN induced the synthesis of immunoreactive PGE and PGA at early and late stages, respectively, of vaccinia virus infection in mouse L fibroblasts. Only species-specific IFN possessed this activity and PG synthesis was stimulated in virus-infected cells, while normal L cells were not affected. The vaccinia virus infection did not significantly alter PG synthesis in the absence of IFN. Indomethacin increased the rate of vaccinia virus replication and partially inhibited the IFN-induced protection of L cells. The addition of exogenous PGA_1 only partially reversed this effect. Finally, short-term PGA treatment induced the synthesis of two enzymes (protein kinase and 2,5A synthetase) thought to be partially responsible for the antiviral action of interferon. These findings suggest that a prostaglandin or PG-related compound seems to mediate at least one aspect of IFN action.

Both IFN and prostaglandins of the A series (PGA) inhibit the replication of vaccinia virus by blocking the synthesis of virus proteins and influencing the control of the expression of specific viral genes in mouse L fibroblasts and HeLa cells (1,2). The similarities suggest a common inhibitory mechanism(s). In the present study, we used the vaccinia virus-(strain WR) mouse L-cell system as a model to investigate whether the antiviral action of prostaglandins and interferon are interrelated. The correlation could conceivably be direct, i.e. PG inducing IFN or vice versa, or indirect, i.e. the two agents inhibit one or more steps of virus replication by similar mechanism(s). The antiviral action of prostaglandins is unlikely to be mediated by the induction of IFN, since PGA's exert their therapeutic effect at a later stage than IFN (successful treatment with PGA can be started 90 minutes after viral infection and in contrast to IFN, pre-treatment of cells is not required). The alternative possibility that IFN could stimulate

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prostaglandin synthesis which in turn might inhibit virus replication was investigated in vaccinia virus-infected cells.

METHODS AND MATERIALS

Mouse L fibroblasts were grown at 37°C as monolayers in Eagle's minimal essential medium (MEM) containing 10% heat-inactivated newborn calf serum (NCS). These cells normally synthesize immunoreactive PGE and PGA, more than 90% of which is secreted into the media. The extracellular PG concentrations were measured in uninfected and virus-infected cells by radioimmunoassay after organic solvent extraction and silicic acid chromatography (3). While PGE synthesis has been demonstrated in many cell lines, PGA synthesis has only been shown in a few cases (4,5) and, with the exception of the coral, plexaura homomalla (6), their formation involves PGE and PG-endoperoxide intermediates. In order to better define the nature of the substance cross reacting with anti-PGA antibody, the samples were separated by high pressure liquid chromatography (HPLC) (7), after concentration of the supernatants during extraction (50 ml of sample from 10^8 cells were concentrated to 200 µl of extraction mixture). The fraction co-migrating with PGA standard was collected and found to immunoreact with anti PGA-antibody. This fraction will be referred to as iPGA (immunoreactive PGA).

In order to study the effects of interferon on PG synthesis, confluent monolayers of L cells were treated with mouse IFN 500 U/ml (sp. act. 4 x 10^7 U/ml) or indomethacin (5 x 10^{-8}M) for 24 hours before infection with 10 p.f.u./cell of vaccinia virus. After one hour of adsorption, virus inocula were removed and MEM + 1% NCS was added. Supernatants were collected at regular intervals and PG levels were determined.

The effects of IFN, PGA, and indomethacin on the induction of protein kinase and 2,5A synthetase were studied. Confluent monolayers of L cells were treated with mouse IFN (500 U/ml, 18 h), indomethacin (5 x 10^{-8}M, 18 h), PGA_1 (4 µg/ml, 3 h) or control diluent.

For determination of protein kinase activity, cells were washed in PBS, scraped into lysis buffer (20 mM Hepes, pH 7.5, 120 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 10% glycerol, 0.5% Nonidet (P-40), centrifuged at 5000 g for 5 min. and the supernatants (250 µl) collected for assay. The presence of the prtein kinase (68,000 dalton) activity was assayed by incubating the extracts (15 µl) in the presence (+) or absence (-) of synthetic double-stranded RNA (400 ng/ml of polyI-poly C), 400 µM ATP and 10 µCi/ml of (γ^{32}P) ATP (2,000 Ci/mmol). After incubation at 30°C for 45 min, the ^{32}P-labeled products were analysed by SDS-PAGE followed by autoradiography.

In the studies of 2-5A synthetase activity, the conditions for preparation of cell extracts were identical. Poly I - poly C-Agarose beads (P.L. Biochemicals) (50 µl) were washed once in 150 µl of buffer B (20 mM Hepes, pH 7.5, 120mM KCl, 5 mM $MgCl_2$, 1mM dithiothreitol, 10% glycerol) containing 0.5% NP-40. Extracts (75 µl) were then mixed, incubated at 4°C for 15 min, washed once in buffer B + 0.5% NP-40 and 3 times in buffer B. The beads were resuspended in 50 µl of buffer B containing 0.5 mM ATP, 100 µCi/ml ^{3}H-ATP (29 Ci/mmol) and incubated at 30°C for 20 h with continous agitation. The beads were sedimented and washed with 25 µl of H_2O and the supernatants pooled. To remove unincorporated ATP, the supernatants were added to 50 µl of DEAE-cellulose equilibrated with buffer B. After washing 5 times with 250 µl of buffer B containing 90mM KCl, oligo 2', 5'A was eluted with 75 µl of 350 mM KCl, 20 mM Tris-HCl, pH 7.5, and quantitated by scintillation counting in Liquiscint (National Diagnostic).

RESULTS

Figure 1 shows the effect of mouse IFN on prostaglandin synthesis in uninfected and WR-infected L cells. In contrast to the fact that mechanical

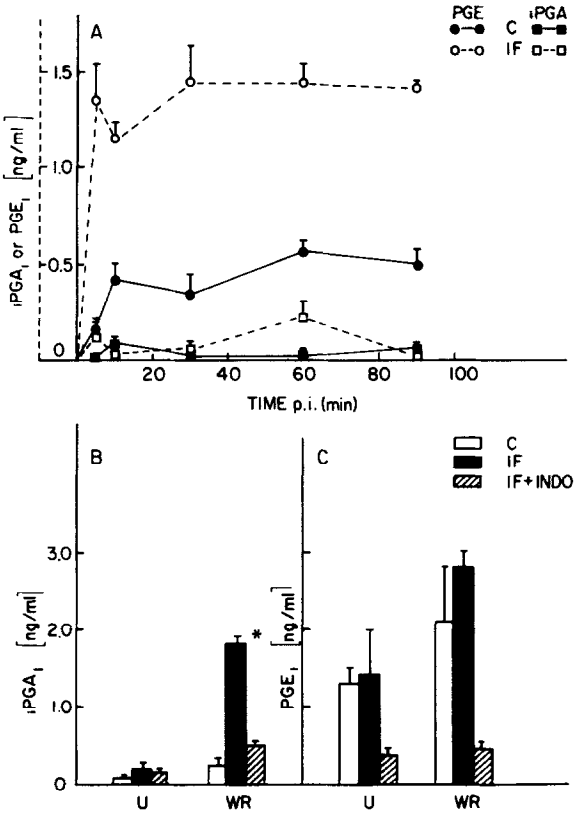

Fig. 1 IFN-mediated stimulation of PG's in vaccinia virus-infected cells. B and C represent studies at 24 hours p.i. Data are expressed as the mean ± S.E.M. of at least duplicate samples. U = uninfected. WR = vaccinia virus-infected.* $p<0.05$.

stimulation of cultured cells generally induces PG synthesis, the alteration of the host cell membrane due to the virus adsorption, entry into the cell and virus maturation, did not significantly activate cellular prostaglandin synthesis. Only a slight increase in PGE could be detected 24 hr postinfection (p.i.) (Figure IC). The absence of prostaglandin synthesis activation might be due to a virus-specific product, since infection with UV-irradiated (9,600 erg/mm^2) inactive WR virus, that penetrates the cell but does not replicate, did stimulate PGE synthesis in the same cells (at 2 hr p.i., C = 3.23 ± 0.12; WR = 1.65 ± 0.24; UV-WR = 5.01 ± 0.76 ng/ml PGE; $p<0.05$).

In uninfected L cells, 24 to 48 hr-treatments with IFN did not stimulate either PGE or iPGA synthesis (Figure IB and C). In WR-infected cells, in contrast, IFN-treatment significantly stimulated PGE synthesis at early times

after infection (Figure IA); at later times (24 hrs) p.i., PGE sythesis was only slightly stimulated (Figure IC), while a significant increase in iPGA production was observed (Figure IB). The same results were obtained in 5 separate experiments. Only mouse-specific IFN was able to stimulate prostaglandins in WR-infected cells (at 24 hrs p.i., C = 1.03 ± 0.07; mouse IFN = 4.55 ± 0.30; human IFN = 1.87 ± 0.49 ng/ml iPGA). Both PGE and iPGA synthesis were strongly inhibited by treatment with $5x10^{-8}$M indomethacin (Indo) (Figure IB,C). In order to determine whether the stimulation of PG synthesis by IFN was necessary for its antiviral action, the effect of PG synthesis inhibitors on virus replication and on IFN action was studied. Table I shows the effect of mouse IFN (M-IFN), human IFN (H-IFN), and indomethacin on vaccinia virus yields. In this experiment, confluent monolayers of L cells were treated with M-IFN, H-IFN or indomethacin for 24 hrs before viral infection. As expected, the antiviral effect was specific for mouse IFN, while indomethacin alone increased WR production. No change in the size of the plaques was noted. The presence of indomethacin during IFN treatment partially prevented the IFN-mediated protection, causing a 600% increase in virus yield. Interestingly, indomethacin treatment p.i. (after one hour of virus adsorption) of IFN-pretreated cells also partially inhibited IFN action (at 24 hrs p.i., C = 1.09 ± $0.09x10^{8}$; M-IFN = 9.8 ± $1.8x10^{6}$; M-IFN+ Indo = 2.68 ± $0.38x10^{7}$; M-IFN+ Indo

TABLE 1

EFFECT OF INDOMETHACIN ON THE ANTIVIRAL ACTION OF IFN

	Vaccinia virus (p.f.u./ml)
Control	$3.56 \pm 0.60 \times 10^{7}$
M-IFN (500 U/ml)	$2.60 \pm 0.39 \times 10^{5}$
H-IFN (500 U/ml)	$4.26 \pm 0.34 \times 10^{7}$
Indomethacin (5×10^{-8}M)	$7.48 \pm 0.48 \times 10^{7}$*
M-IFN + Indomethacin	$1.82 \pm 0.06 \times 10^{6}$**

Mouse L cells were infected for one hour at 37°C with 10 p.f.u./cell of purified vaccinia virus (strain WR) and the virus yields at 24 h p.i. were titrated on Vero cells (10). Data are expressed as the mean ± S.E.M. of at least quadruplicate samples (M-IFN=mouse IFN; H-IFN = human IFN).

*$p<0.01$ vs. control; **$p<0.01$ vs. M-IFN

+ PGA_1 (4 μg/ml) = 1.96 ± $0.43x10^7$ WR pfu/ml). The addition of exogenous PGA partially but incompletely restored IFN protection, which indicates that either another PG metabolite is required to overcome the inhibitory effect of indomethacin or, as has been previously suggested (8), cyclooxygenase activity per se is necessary for complete IFN antiviral action. Since inhibition of PG synthesis only partially prevented the antiviral action of IFN, IFN must have been acting through at least two separate mechanisms, only one of which is related to PG synthesis.

Since the antiviral action of interferon has been shown to be correlated with the induction of two ds-RNA dependent enzymes, a protein kinase and a 2,5A synthetase (9), we evaluated whether PGA had any effect on the induction of these enzymes. Figure 2 shows that 3 hour treatment of confluent L cells with PGA_1 (4 μg/ml) significantly stimulated the synthesis of both the protein kinase and the 2,5A synthetase, even though to a lesser extent than IFN.

Figure 2 also shows that indomethacin treatment, previously reported to almost completely suppress the IFN-mediated induction of another enzyme, indoleamine 2,3-dioxygenase, in mouse lung slices (10), did not significantly alter the synthesis of the protein kinase and 2,5A synthetase. Since indomethacin reduced the antiviral action of IF, its lack of effect on the kinase and synthetase suggests that these enzymes are not solely responsible for the antiviral action in this system.

DISCUSSION

In the past few years, interferon (IFN) and prostaglandins (PG's) have been shown to possess similar regulatory properties, influencing the immune response, cell proliferation and differentiation, and virus replication (11-13). The possibility of a relationship between PG's and IFN action has recently been the subject of many studies. Yaron *et al* (14) demonstrated that IFN itself as well as an inducer of IFN-, poly (rI). poly (rC), stimulated prostaglandin E (PGE) synthesis in foreskin and synovial fibroblasts, and the time course, dose dependence and nucleotide specificity are similar to that of IFN induction. Similarly, other inducers of IFN, such as RNA and DNA

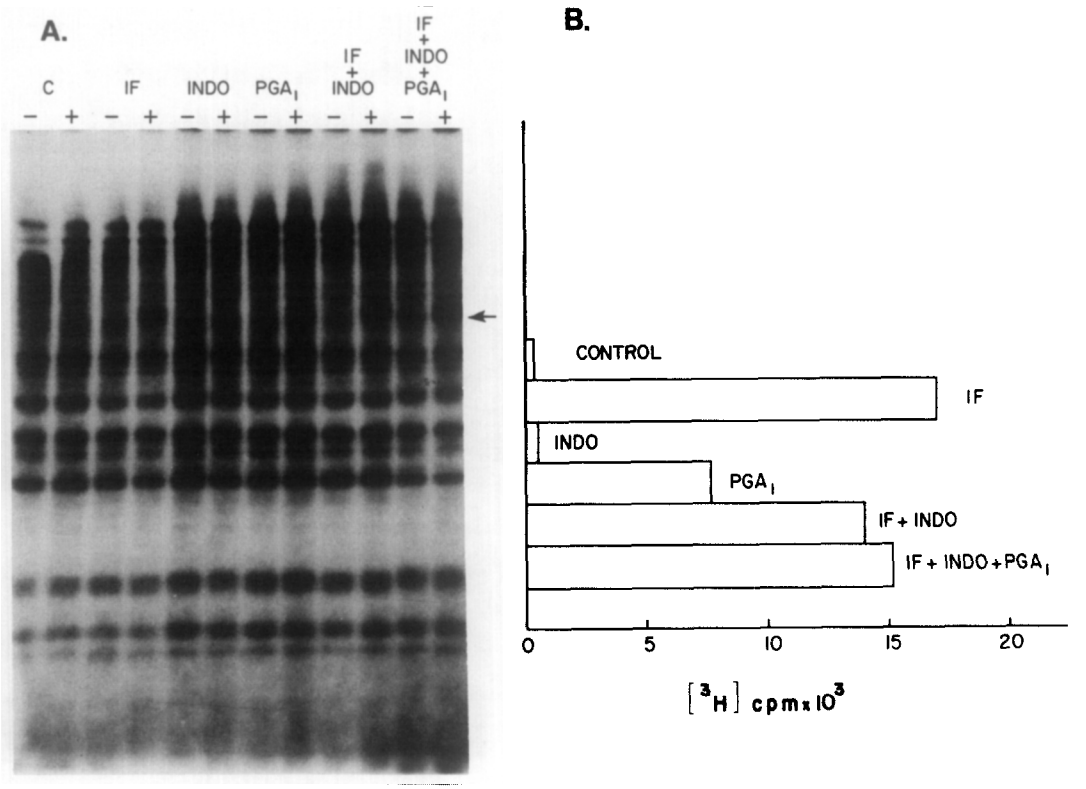

Fig. 2 The effect of IFN, PGA, and indomethacin on the induction of protein kinase (A) and 2, 5A synthetase (B).

viruses, antigens and mitogens, can also induce PGE synthesis (15-17). Interferon induction correlates closely with the increased cellular PG production in seven distinct cell lines, infected with six viruses (18), and prostaglandins of the A, E and F series have been reported to restore the interferon response in hyporeactive animals (19). Moreover, inhibitors of prostaglandin synthesis have been shown to reduce the antiviral action of interferon (8), and the resistance to both the antiviral and antitumor properties of mouse interferon in a clone of L1210 mouse leukemia cells was found to be coupled to the absence of cyclooxygenase activity (20).

The observations in this study suggest that a prostaglandin or PG-related compound might influence of interferon's antiviral action; however, the fact that indomethacin only partially inhibited IFN action and that this effect could not be reversed by the addition of exogenous prostaglandins indicates that IFN could be acting through several mechanisms, only one of which is

related to PG's. We propose that this common mechanism is likely to be at the transcriptional and/or translational level, since as shown in this study, both agents induce the synthesis of specific antiviral enzymes and both have been shown to inhibit the synthesis of specific vaccinia virus proteins (although it is not known if the proteins suppressed are identical). This hypothesis and the fact that IFN is known to be a pleiotropic effector (21) could account for the contradictory results reported on the effect of PG synthesis inhibitors on the antiviral action of IFN in different virus-cell systems.

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