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COMPARATIVE EFFECTS OF INTERLEUKIN 1 AND A PHORBOL ESTER ON RHEUMATOID SYNOVIAL CELL FRUCTOSE 2,6-BISPHOSPHATE CONTENT AND PROSTAGLANDIN E PRODUCTION

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The addition of either recombinant human interleukin 1 (IL1α) or 12-O-tetradecanoyl phorbol-13-acetate (TPA) to cultured rheumatoid synovial cells (RSC) caused dose-related increases in PGE production and cellular fructose 2,6-bisphosphate (Fru-2,6-P_2). IL1 consistently produced the greater increases in both parameters. A close association between increases in PGE production and Fru-2,6-P_2 was demonstrated for both IL1- and TPA-stimulated cells. The combined addition of IL1 with TPA resulted in an additive increase in both parameters. When IL1 was added together with human recombinant interferon-gamma (IFN-γ) , the resulting Fru-2,6-P_2 level was synergistically increased, whilst the combination of IFN-γ and TPA produced only an additive increase. Thus despite their very similar effects on RSC in culture, the data suggests that IL1 and TPA do not act via an identical intracellular mechanism.

The cytokine interleukin 1 (IL1) induces a wide range of effects in cultured rheumatoid synovial cells (RSC), including increases in prostanoid, proteinase and glycosaminoglycan synthesis (1-4), although the intracellular events which link IL1 receptor occupancy to these actions is as yet unknown. IL1 also stimulates RSC proliferation, but this can usually be demonstrated in vitro only in the presence of an inhibitor of prostaglandin synthesis (2). Increased glycolysis is one of the early responses observed in mitogen-stimulated fibroblasts and we have recently confirmed that both IL1 and interferon - γ (IFN-γ) increase glycolysis of cultured RSC (5). This enhanced metabolism may be mediated in part via the important glycolytic regulatory metabolite fructose 2,6-bisphosphate (Fru-2,6-P_2) which increases several fold in RSC exposed to IL1 and IFN-γ in parallel with other increased parameters of glycolysis (5).

Synovial cell proliferation, prostanoid and proteinase production are all markedly stimulated by the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) (6), which also increases Fru-2,6-P_2 in chick embryo fibroblasts (7). TPA specifically activates protein kinase C an enzyme involved in transmitting information from the cell membrane to regulate many cellular functions and proliferation (8). The similar patterns of cellular response to IL1 and TPA, suggest that the IL1 effects on RSC might be

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mediated , like other growth factors (8), by protein kinase C activation. We have examined this hypothesis by studying the effects of IL1 and TPA, either singly or together, on RSC Fru-2,6-P_2 and PGE production, in the presence and absence of IFNγ.

MATERIALS AND METHODS

Synovial cell culture

Fresh rheumatoid synovium obtained from remedial synovectomies or knee replacement operations was dissociated enzymatically as described previously (1). Adherent cells were cultured in Dulbecco's modified Eagles medium (DMEM) containing 10% foetal calf serum (FCS) and antibiotics in 6 or 12-well plastic cluster dishes (Costar). Cultures were incubated in a water-saturated atmosphere of 5% CO_2 in air. The adherent rheumatoid synovial cells (RSC) of the primary culture contained variable proportions of fibroblasts, macrophages and dendritic cells, but after subculture the passaged cells exhibited a uniformly fibroblastic appearance. Three subcultured preparations of RSC were used for these experiments after various times in culture between first- and seventh-passage. Monolayers of RSC approaching confluence were incubated with DMEM/10% FCS with and without cytokines or phorbol ester for either 7 or 18h. The medium was then collected and subsequently assayed for PGE using conventional radioimmunoassay (5). The cells were either solubilized with 50mM NaOH/1% Triton X-100 for Fru-2,6-P_2 measurement (7) or precipitated with 6% (w/v) $HClO_4$ for protein estimation by the method of Lowry et al (9). Although the total cell protein per well showed variation with different cell cultures, within a single experiment the variation in cell protein/culture well was consistently less than 5%.

To measure Fru-2,6-P_2 the solubilized cells were scraped into tubes and heated for 10min at 80°C to denature protein and stabilise Fru-2,6-P_2. After cooling in an ice-water bath the samples were made 20mM in Hepes, adjusted to neutral pH with 1M-acetic acid and centrifuged (9000g, 2min). Fru-2,6-P_2 was assayed in the supernatant by the method of Van Schaftingen at al (10) as modified by Van Schaftingen and Hers (11).

Recombinant human IL1 α with a specific activity of 4×10^7 units/mg in the thymocyte co-stimulation assay (12) was a gift from Roche Products Ltd, U.K. Recombinant human IFN-γ with a specific activity at 2×10^7 antiviral units/mg was a generous gift of Boehringer Ingelheim, Vienna, Austria. Recombinant human IL1β (Cistron Technology, USA) units of activity were defined by the same assay system as the IL1$^{\alpha}$. Since TPA (Sigma) was dissolved in dimethyl sulphoxide (1mg/ml) before adding to the culture media, we confirmed that the appropriate concentration of solvent did not affect RSC Fru-2,6-P_2 content or PGE production.

RESULTS AND DISCUSSION

IL1 $^{\alpha}$ and TPA individually caused dose-related increases in RSC Fru-2,6-P_2 and PGE production (Fig. 1a,b). An IL1 α concentration of one unit/ml produced the maximal rise in both parameters, whilst for TPA no further increase occurred above a concentration of 10ng/ml. A linear regression analysis of the results in Fig. 1a,b for PGE production and Fru-2,6-P_2 gave the following correlation coefficients: 0.96 (n=15) for control and IL1 - treated cells; 0.95 (n=15) for control and TPA-treated cells; 0.93 (n=27) for

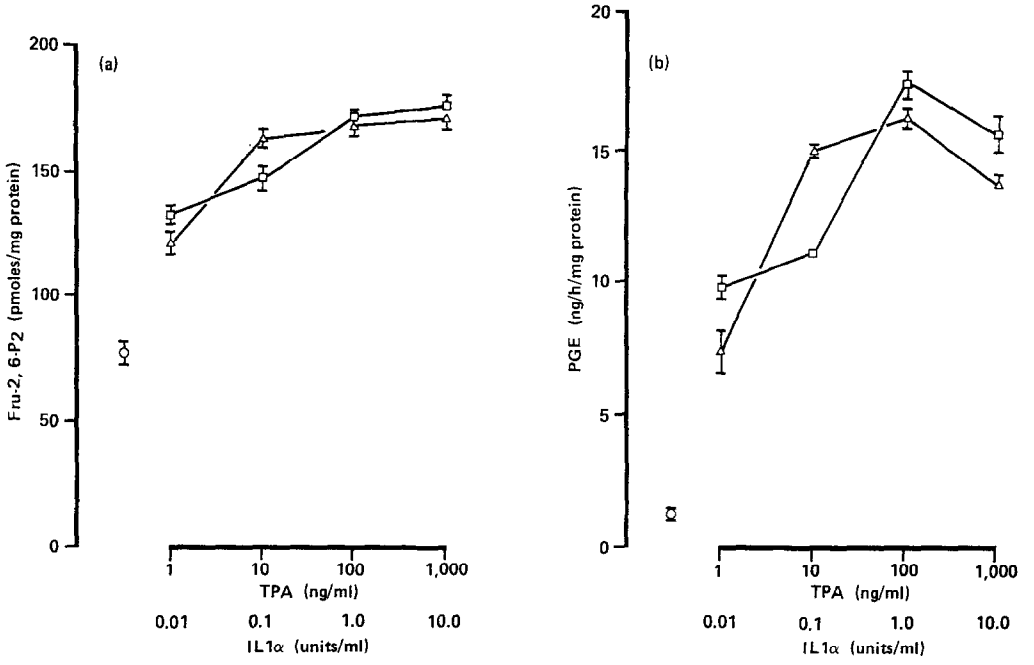

Figure 1. Effect of TPA and IL1α on RSC Fru-2,6-P_2 content and PGE production.

RSC were incubated with DMEM-FCS, (O); TPA (Δ); IL1α (□); for 7 hours. The medium was removed and assayed for PGE production (b) whilst the cells were processed for Fru-2,6-P_2 measurement (a) as described in the Methods. Values are mean ± S.E.M. (n = 3).

all treatments. This close association between these parameters was unaffected by increasing the incubation times from 7 to 18h and was a feature of cells stimulated by either IL1 α or IL1 β (result not shown). In eight experiments on three different cell preparations IL1 consistently achieved a greater maximal level ($P < 0.01$ paired t-test) of both Fru-2,6-P_2 and PGE production than did TPA (Table 1).

The combination of TPA (at a fixed concentration of 100ng/ml) with varying concentrations of IL1 resulted in an approximately additive increase in both Fru-2,6-P_2 and PGE production further demonstrating the close association between both responses (Fig. 2a,b). Thus when TPA was added with concentrations of IL1 of 1 unit/ml and above, the resulting values for both parameters were greater than could be achieved by either single mitogen. This additive effect could be demonstrated over a range of IL1 concentrations (Fig. 2a,b) and also with the β form of IL1 (result not shown).

When IFN-γ, which alone increased RSC Fru-2,6-P_2 approximately two fold, was added with IL1 or TPA the effect on Fru-2,6-P_2 differed with each mitogen. IL1 α and IFN-γ produced a synergistic increase in the cellular metabolite, whilst TPA and IFN-γ produced only an additive increase (Table 2). Despite augmenting cellular Fru-2,6-P_2, IFN-γ did not increase RSC PGE production when added singly or in combination with IL1 α or TPA, thus

TABLE 1. MAXIMAL LEVELS OF RSC FRU-2,6-P_2 AND PGE PRODUCTION ACHIEVED BY TPA AND IL1 α

	PGE (ng/h/mg protein)			Fru-2,6-P_2 (pmoles/mg protein)		
Cells	Control	TPA	IL1	Control	TPA	IL1
RSC 1 p4	< 0.7	8.5 ± 0.2	14.2 ± 0.2	73 ± 7	159 ± 8	227 ± 7
RSC 1 p6	< 0.1	11.3 ± 0.3	15.5 ± 0.6	51 ± 1	88 ± 3	151 ± 6
RSC 2 p1	1.3 ± 0.1	14.9 ± 0.2	17.4 ± 0.5	76 ± 5	155 ± 4	170 ± 4
RSC 2 p2	1.9 ± 0.1	26.2 ± 1.2	32.5 ± 1.6	86 ± 2	179 ± 4	216 ± 6
RSC 3 p6	< 0.8	14.5 ± 1.9	32.6 ± 2.0	62 ± 7	156 ± 7	218 ± 12
RSC 2 p2	< 0.2	13.2 ± 0.1	16.2 ± 0.3	81 ± 3	150 ± 6	258 ± 3
RSC 3 p7	< 0.8	19.2 ± 0.7	34.1 ± 1.5	93 ± 7	141 ± 6	243 ± 5
RSC 2 p2	0.3 ± 0.1	9.3 ± 0.4	15.9 ± 0.1	100 ± 9	222 ± 6	241 ± 3

RSC were incubated with DMEM-FCS ± TPA (10ng/ml) or IL1 α (1 unit/ml) for 7 or 18 hours. The medium was removed and assayed for PGE production and the cells processed for Fru-2,6-P_2 measurement as described in the Methods. Values are mean ± S.E.M. (n = 3).

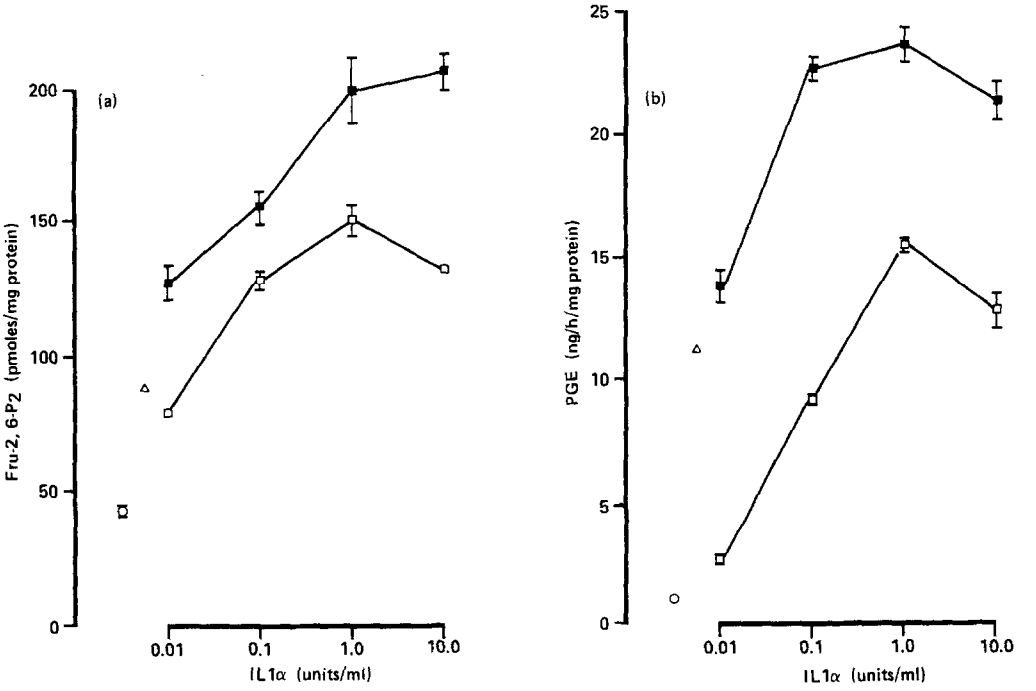

Figure 2. Effect of combined addition of TPA and IL1α on RSC Fru-2,6-P_2 and PGE production

RSC were incubated with DMEM-FCS, (○); TPA-100ng/ml, (△); IL1 α, (□); IL1 + TPA-100ng/ml, (■); for 7 hours. The medium was removed and assayed for PGE production (b) whilst the cells were processed for Fru-2,6-P_2 measurement (a) as described in the Methods. Values are mean ± S.E.M. (n = 3).

Table 2. EFFECT OF COMBINED ADDITION OF IFN-γ WITH TPA OR IFN-γ WITH IL1α ON RSC FRU-2,6-P_2 CONTENT

Treatment	Fru-2,6-P_2 (pmole/mg protein)	PGE (ng/h/mg protein)
DMEM-FCS	100 ± 9	0.3 ± 0.1
IFN-γ (70u/ml)	215 ± 0	< 0.2
TPA (10ng/ml)	222 ± 6	9.3 ± 0.4
IL1α (10u/ml)	241 ± 3	15.9 ± 0.1
IFN-γ + TPA	277 ± 25	6.5 ± 0.5
IFN-γ + IL1 α	536 ± 14	11.9 ± 0.2

RSC were incubated with DMEM-FCS ± mitogen(s) for 18 hours. The medium was removed and assayed for PGE production and the cells processed for Fru-2,6-P_2 measurement as described in the Methods. Values are mean ± S.E.M. (n = 3).

providing the single exception to the otherwise close association between Fru-2,6-P_2 and PGE production noted in these studies. (result not shown).

The present dose-response study confirms our earlier observations (5) that a concentration of IL1 above one unit/ml produces no further increase in RSC Fru-2,6-P_2 or PGE production. The corresponding concentration of TPA (10ng/ml) was the same as that reported to increase rabbit synovial fibroblast PGE production (6) and Fru-2,6-P_2 content in chick embryo fibroblasts (7). Thus the previously observed similar patterns of IL1 and TPA induced responses in synovial fibroblasts have been confirmed and extended in the present experiments, further supporting the concept of a similar intracellular mechanism. The close correlation between PGE production and cellular Fru-2,6-P_2 in both IL1- and TPA-stimulated cells suggested that the increase in glycolysis might be a result of increased prostanoid metabolism. However, this is not the case since both dexamethasone and NSAIDs prevent the rise in PGE production but not Fru-2,6-P_2 (5).

In the present investigations, IL1α (and IL1β), consistently produced a greater maximal stimulation of Fru-2,6-P_2 and PGE production than did TPA. There are several possible explanations for this difference. Assuming that TPA can produce maximal stimulation of protein kinase C activity our findings suggest that IL1 does not act via the same kinase or, if it does, it must have additional components to its signal. The second alternative, whereby only a part of the IL1 signal involves activation of the TPA-stimulated protein kinase C, seems unlikely in the face of the results obtained when IL1 and TPA were added in combination. This resulted in an additive increase in both cellular parameters, even at a concentration of TPA which produced the maximal response when added alone. If the two mitogens shared any significant part of their signal mechanism only a small increase in these parameters would have been predicted and certainly not the additive effect obtained. Our observations would be explicable on the basis of IL1 acting via a different intracellular mechanism to TPA.

Further evidence for this hypothesis was provided in the experiments in which each mitogen was added with IFN-γ ,which we have previously shown

increases Fru-2,6-P_2 but not PGE production (5). When IFN-γ was added with IL1 α or β the resulting cellular Fru-2,6-P_2 was synergistically increased, whilst for TPA and IFN-γ the increase was no more than additive. The reason for the synergism between IL1 and IFN-γ is unknown, but it suggests an interaction between the cellular signals produced by these cytokines at a level prior to activation of the enzyme which produces Fru-2,6-P_2. In contrast, the additive increase in Fru-2,6-P_2 resulting from the combinations of TPA with IL1, or TPA with IFN-γ suggests that these pathways come together at a later stage in signal transmission.

Thus IL1, TPA and IFN-γ have at least one property in common, the ability to increase RSC Fru-2,6-P_2 with an associated rise in glycolytic flux (5). The cellular response to TPA and IL1 is however clearly different from that to IFN-γ the latter being unable to stimulate PGE production. For TPA and IL1 other reported differences in the resulting cellular responses are more subtle, having so far been confined to dissimilarities in the molecular weights of proteins phosphorylated following exposure to the mitogens (13). Our experiments show that further differences exist and it now seems most unlikely that even a part of the intracellular signal generated by IL1 on RSC involves the TPA-activatable protein kinase C.

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