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PHORBOL ESTERS ACTIVATE PROTEIN KINASE C AND GLUCOSE TRANSPORT AND CAN REPLACE THE REQUIREMENT FOR GROWTH FACTOR IN INTERLEUKIN-3-DEPENDENT MULTIPOTENT STEM CELLS

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SUMMARY

Interleukin 3 (IL-3) promotes the survival, proliferation and development of progenitor cells from several distinct haemopoietic lineages and can also stimulate the self-renewal of stem cells. We have explored the mode of action of this growth factor in promoting survival and proliferation, using a multipotent haemopoietic stem cell line FDC-Mix 1. In the absence of IL-3 these cells died within 16–48 h. However, this requirement for IL-3 could be replaced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) plus Ca^{2+} ionophore, which promoted not only survival but also DNA synthesis with no concomitant loss of the multipotential nature of these cells. TPA and Ca^{2+} ionophore, respectively, could also interact synergistically with IL-3 to promote DNA synthesis. Both IL-3 and TPA stimulated the translocation of protein kinase C (PK-C) from the cytosol to a membrane-bound form in FDC-Mix 1 cells. Previously we suggested that IL-3 can activate the primary metabolism of IL-3-dependent cells so that increased glucose transport and glycolysis lead to maintenance of ATP levels and cellular survival. To investigate whether TPA and, or, Ca^{2+} ionophore could also influence cellular survival *via* an activation of glucose uptake we assessed the effects of these agents on hexose transport. TPA ± Ca^{2+} ionophore activated hexose transport to the same degree as does IL-3 but these agents cannot superstimulate FDC-Mix 1 hexose transport in cells that already exhibit an activated transport system from preincubation with IL-3. We conclude that IL-3 maintains FDC-Mix 1 cells *via* its ability to activate PK-C and increase cytosolic levels of Ca^{2+}, and that an IL-3-mediated activation of PK-C may promote cellular survival *via* its ability to enhance hexose uptake by phosphorylating the glucose transport protein.

INTRODUCTION

All mature haemopoietic cells are derived from primitive stem cells that persist throughout life because of their ability to undergo self-renewal. Stem cells are also multipotent and can undergo differentiation into committed cells, which then go on to further proliferate and develop into the mature cells. Analysis of haemopoietic cell

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development *in vitro* has revealed that cell survival, proliferation and maturation may be controlled, at least in part, by a series of haemopoietic growth factors (Burgess & Nicola, 1983). Some of these growth factors display a target cell restriction (e.g. macrophage colony-stimulating factor or CSF-1 (Stanley & Jubinsky, 1984), granulocyte colony-stimulating factor (Nicola *et al.* 1983) and erythropoietin (Eaves & Eaves, 1984)) and can only support the survival, proliferation and development of progenitor cells committed to a specific haemopoietic lineage. Other growth factors, however, seem able to stimulate the development of several cell lineages. These include granulocyte/macrophage colony-stimulating factor, which promotes the growth and development of neutrophils, macrophages and eosinophils (Gough *et al.* 1984), and interleukin 3 (IL-3), a murine haemopoietic growth factor, which facilitates the survival, proliferation and development of progenitor cells of the megakaryocytic, erythroid, granulocytic, macrophage and mast cell lineages. Significantly, IL-3 can also promote the self-renewal and development of multipotential stem cells (Bazill *et al.* 1983).

The variety of primitive cells that are stimulated by IL-3 indicates that this molecule may be of some physiological significance in the process of early haemopoietic cell development. Thus the biochemical events elicited by this growth factor are obviously of fundamental importance in our understanding of self-renewal and development. To approach this problem, we have studied the response, to IL-3, of a variety of cell lines that absolutely require this growth factor for their survival and proliferation. These cells die within 12–48 h in the absence of IL-3, a phenomenon that we have attributed to the ability of IL-3 to promote the primary metabolism of these cells leading to increased glucose uptake and ATP synthesis (Whetton *et al.* 1984).

The further question of how IL-3 generates a signal within the cell to activate cellular metabolism, survival and proliferation remains unanswered. To explore the possible 'messenger' molecules, which are generated by IL-3 to stimulate these events, it is first necessary to establish a *biological* effect of those messenger molecules on the target cell population, i.e. any attempts to demonstrate an IL-3-mediated second messenger production must be accompanied by ample evidence of a biological function for those second messengers within the cell. For this reason we have studied the ability of agents that can mimic the generation of second messengers *via* phosphatidylinositol metabolism to achieve biological effects within a self-renewing multipotent stem cell line.

MATERIALS AND METHODS

Cell culture

The FDC-Mix 1 (Factor Dependent Cell-Mix) were originally derived from long-term bone marrow cultures treated with an *src* oncogene-containing retrovirus (Spooncer *et al.* 1984). The cells have a diploid karyotype and are non-leukaemic. Furthermore, the cells do not contain integrated *src* virus and possess only low levels of c-*src* kinase activity (unpublished data). FDC-Mix 1 cells have some characteristics of 'stem' cells in that they can either self-renew or develop along several distinct haemopoietic lineages (i.e. they are multipotent). FDC-Mix 1 cells were

routinely maintained in Fischer's medium (Gibco) plus 20% (v/v) horse serum (Flow Laboratories). WEHI-3B cell conditioned medium (10%, v/v) was added as a source of interleukin 3, which is essential for the survival and proliferation of FDC-Mix 1 cells. Cells were routinely subcultured twice weekly to give between 5×10^4 and 10^5 cells per ml.

FDC-Mix 1 cellular survival and DNA synthesis

To determine the effects of IL-3 and other additives on FDC-Mix 1 cellular survival and DNA synthesis, the FDC-Mix 1 cells were centrifuged at 800 ***g*** for 10 min, the supernatant was removed and the pellet resuspended in Fischer's medium. The cells were then centrifuged again, resuspended and after one further centrifugation (to remove residual IL-3 activity) the cells were resuspended to $10^6\,ml^{-1}$ in Fischer's medium plus 10% (v/v) horse serum. The cell suspension (0·25 ml) plus Fischer's medium supplemented with 10% (v/v) horse serum plus any additives were then added to 24-well Costar tissue culture plates to a final volume of 1·0 ml per well, and the plates were then incubated at 37°C for 24 h.

After this time, samples were removed and the cells were counted in the presence of Trypan Blue to determine the number of viable cells in each incubation. These values are expressed in the text as a percentage of the original inoculum in each microwell. To determine the rate of DNA synthesis the microwell cultures were gently mixed and 100 μl of each incubation was removed and placed in a 96-well tissue culture plate (Costar) to which 10 μl of a $10\,\mu Ci\,ml^{-1}$ [^3H]thymidine solution (Amersham International) was added. After 4 h incubation at 37°C, the cells were harvested and the trichloroacetic acid (TCA)-precipitable material was collected and taken for liquid scintillation counting. The ^3H present in the TCA-precipitable material was used as a relative measure of DNA synthesis.

CFC-Mix assays

Cells from microwell cultures were centrifuged, and resuspended in a CFC-Mix assay medium containing IL-3, erythropoietin and other additives as previously decribed (Spooncer *et al.* 1984). After 9 days the colonies present in the soft agar were counted using an Olympus dissecting microscope at ×21 magnification, and their morphology was determined after isolating individual colonies and staining appropriately (Spooncer *et al.* 1984).

Protein kinase C assays

Protein kinase C activity in membrane and cytosolic fractions was determined using the methods described by Farrar *et al.* (1985) for interleukin-3-dependent cells.

Hexose transport assays

To prepare cells for hexose transport assays, FDC-Mix 1 cells were washed as described above and then resuspended to a concentration of $5\times10^5\,ml^{-1}$ in Fischer's medium plus 10% (v/v) horse serum plus any further additives. Cells were then incubated for 4 h at 37°C after which they were centrifuged (1000 ***g*** × 5 min) and resuspended in ice-cold buffer consisting of 8·1 mM-Na_2HPO_4, 1·4 mM-KH_2PO_4, 2·6 mM-KCl, 136 mM-NaCl, 0·5 mM-$MgCl_2$ and 0·9 mM-$CaCl_2$, final pH 7·4. Cells incubated for 4·0 h without IL-3 were still 100% viable as determined by Trypan Blue exclusion. At this stage, cells were preincubated for 4 min at 37°C with other additives, after which the uptake of 2-deoxyglucose or L-glucose was measured as described by Whetton *et al.* (1984) except that 10^6 to 2×10^6 cells were used per assay.

Interleukin 3 (IL-3)

The IL-3 used in these experiments was a highly purified (a single band on silver-stained sodium dodecyl sulphate (SDS)–polyacrylamide electrophoresis gels) preparation, which was maintained in a carrier protein solution of 1% (w/v) bovine serum albumin. IL-3 activity was determined as described by Bazill *et al.* (1983), using the FDC-P2 cell line.

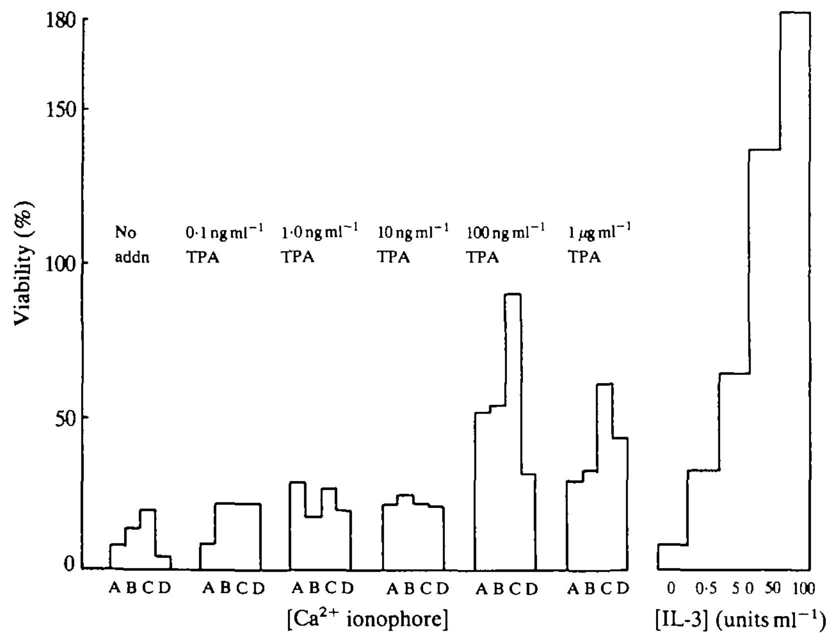

Fig. 1. Effect of interleukin 3, TPA and Ca^{2+} ionophore on FDC-Mix 1 cell survival. Results shown are the means of three observations from one experiment. Five such experiments were performed giving similar results to those shown above. A. No Ca^{2+} ionophore added; B, $+Ca^{2+}$ ionophore ($10\,ng\,ml^{-1}$); C, $+Ca^{2+}$ ionophore ($100\,ng\,ml^{-1}$); D, $+Ca^{2+}$ ionophore ($1{\cdot}0\,\mu g\,ml^{-1}$).

RESULTS

Survival and proliferation of FDC-Mix 1 cells

FDC-Mix 1 cells are absolutely dependent on interleukin 3 for their survival and proliferation; in its absence they die within 12–48 h. Removal of the growth factor for 14 h is sufficient to lead to the irreversible decline of FDC-Mix 1 cells such that readdition of large quantities of IL-3 cannot recover these cells. This dependence on IL-3 is shown in Fig. 1 and is similar to the effect observed with other IL-3-dependent cell lines. Previously we have suggested that this IL-3 effect occurs *via* activation of the primary metabolism of the cells, associated with the generation of ATP (Whetton *et al.* 1984). Certainly, the IL-3-mediated survival is dose-dependent and is associated with the specific binding of the growth factor to a $70\,000 M_r$ interleukin-3 receptor found on the surface of FDC-Mix cells (A. W. Burgess & T. M. Dexter, unpublished observations). How does IL-3 receptor occupation lead to cellular survival and proliferation, and what intracellular signals are generated to achieve these effects within the cell? We have shown that agents that increase cyclic AMP levels are unable to replace IL-3 as a survival signal (Whetton & Dexter, 1983). Here we have examined the ability of agents that can replace the second messengers generated by phosphatidylinositol metabolism, namely 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) and Ca^{2+} ionophore (or A23187) (Berridge & Irvine, 1984). At optimal concentrations of these two agents ($100\,ng\,ml^{-1}$ of each) the survival of cells in the absence of IL-3 is increased from 5 % to 75–95 % of the cells originally seeded

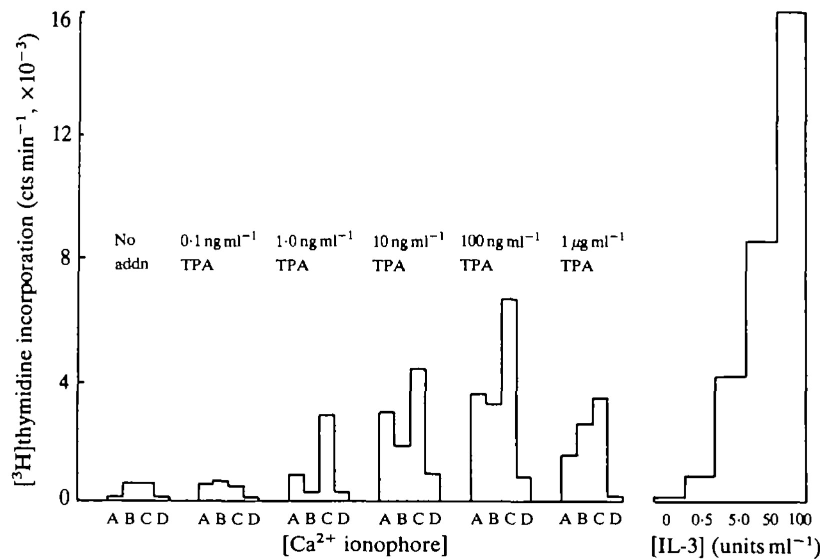

Fig. 2. Effect of interleukin 3, TPA and Ca^{2+} ionophore on FDC-Mix 1 cell thymidine incorporation. Results shown are the means of four observations from one experiment. Five such experiments were performed giving results similar to those shown above. A. No Ca^{2+} ionophore added; B, $+Ca^{2+}$ ionophore (10 ng ml^{-1}); C, $+Ca^{2+}$ ionophore (100 ng ml^{-1}); D, $+Ca^{2+}$ ionophore (1·0 μg ml^{-1}).

(Fig. 1). This corresponds to about 25 units ml^{-1} of interleukin-3 activity. Furthermore, DNA synthesis is also markedly enhanced by TPA+Ca^{2+} ionophore (both 100 ng ml^{-1}) (Fig. 2). However, the effects of IL-3 alone, TPA, or TPA plus Ca^{2+} ionophore, are totally reversible by washing the FDC-Mix 1 cells to remove any additives; without IL-3 the cells then died. But treatment of the cells with TPA plus Ca^{2+} ionophore followed by washing the cells and incubating with IL-3 led to their continued survival and proliferation.

Maintenance of FDC-Mix 1 cell multipotentiality by TPA plus Ca^{2+} ionophore

We have also assessed whether FDC-Mix 1 cells incubated with TPA plus Ca^{2+} ionophore retain their multipotential nature in the CFC-Mix assay. FDC-Mix 1 cells can be induced to differentiate to form colonies of mixed myeloid cells in soft agar, demonstrating the multipotential nature of this cell line (Dexter *et al.* 1985). Treatment with Ca^{2+} ionophore and TPA does not inhibit or decrease this ability (Table 1).

Synergistic effects of interleukin 3, TPA and Ca^{2+} ionophore on DNA synthesis

The ability of TPA and Ca^{2+} ionophore to 'replace' (at least in part) IL-3 as a survival/proliferation signal, thereby maintaining a multipotential cell population suggests that IL-3 can also activate protein kinase C (as does TPA) and increase cytosolic levels of Ca^{2+} (as does Ca^{2+} ionophore). If this is the case then IL-3, TPA and Ca^{2+} ionophore, respectively, should interact synergistically as does TPA when added with Ca^{2+} ionophore (Figs 1, 2). Using thymidine incorporation as a measure

of synergism, we used three different concentrations of IL-3 with TPA, Ca^{2+} ionophore, and TPA+Ca^{2+} ionophore, to assess their relative effects on FDC-Mix 1 cells (Fig. 3). At low concentrations of IL-3 (10 units ml^{-1}) TPA and Ca^{2+} ionophore, respectively, acted with interleukin 3 to produce a synergistic increase in DNA synthesis (Fig. 3). However, TPA+Ca^{2+} ionophore + interleukin 3 (10 units ml^{-1}) gave only an *additive* increase in DNA synthesis. Similarly at 50 units ml^{-1} of IL-3, TPA and Ca^{2+} ionophore, respectively, interact synergistically with the growth factor, but TPA+Ca^{2+} ionophore and IL-3 (50 units ml^{-1}) are *less* than additive. The effects of TPA, Ca^{2+} ionophore, and TPA+Ca^{2+} ionophore, at an IL-3 concentration of 100 units ml^{-1} are also *less* than additive. Thus at low concentrations IL-3 will act synergistically with either TPA or Ca^{2+} ionophore and additively with TPA+Ca^{2+} ionophore. At higher concentrations of IL-3 the synergism *and* the additive effects are lost. These results are consistent with IL-3 activating protein kinase C and increasing levels of cytosolic Ca^{2+}, leading to DNA synthesis. However, it must be remembered that a high level of cytosolic Ca^{2+} can itself activate protein kinase C (Nishizuka, 1984).

Translocation of protein kinase C by interleukin 3

In order to validate the putative activation of protein kinase C by IL-3, it is necessary to show such an effect occurs in FDC-Mix 1 cells. It has already been reported (Farrar *et al.* 1985) that IL-3 can mediate an activation (or translocation to membrane sites) of protein kinase C in FDC-P1 cells (a granulocytic precursor cell line; Dexter *et al.* 1980). Using the same procedure as Farrar *et al.* (1985), we also observed an IL-3(and also TPA)-mediated translocation of protein kinase C in FDC-Mix 1 cells (Table 2). The degree of this translocation is not as marked as that

Table 1. *Effect of interleukin 3 and TPA plus Ca^{2+} ionophore on FDC-Mix cell clonogenicity and multipotency*

Pretreatment	Colonies formed in CFC-Mix assay (colonies/1000 cells plated)
No addition*	0
+Interleukin 3 (100 units ml^{-1})	112
+TPA+Ca^{2+} ionophore (both 100 ng ml^{-1})	121

Cells incubated, in the conditions described above + 10 % horse serum, in Fischer's medium for 48 h were centrifuged, washed and 1000 viable cells per Petri dish were replated into CFC-Mix assay medium (see Materials and Methods) and the resultant colonies were counted after 9 days. The colonies were of mixed morphology, erythroid megakaryocytic, granulocytic and macrophage cell types all being present. Results above are the means of at least three observations; standard deviations for the above values were all <11 %.

* To obtain viable cells after 48 h in the absence of interleukin 3 is not possible. This control therefore represents the total number of viable cells remaining in 300 μl culture of $2{\cdot}5 \times 10^5$ cells ml^{-1} plated out into three Petri dishes in each experiment. This is <1 % of the original number of cells seeded.

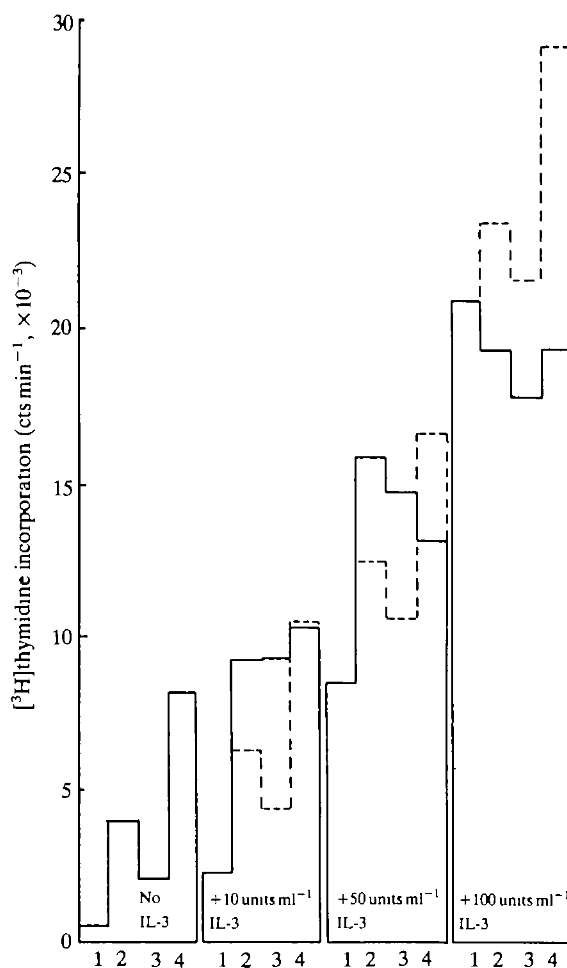

Fig. 3. Effect of Ca^{2+} ionophore, TPA, and interleukin 3 on thymidine incorporation in FDC-Mix 1 cells. Results shown are the means of at least six experiments; standard deviations in all cases were <10%. Where FDC-Mix 1 cells have been treated with two agents the effect of these agents on thymidine incorporation when added *individually* to FDC-Mix 1 cells has been added together and is displayed on the figure as a broken line. This enables the *additive* effects of the two agents on thymidine incorporation to be shown in juxtaposition to the observed experimental observations. TPA and Ca^{2+} ionophore were both added to give a final concentration of 100 ng ml^{-1}. 1. FDC-Mix 1 cells with no additives; 2, FDC-Mix 1 cells + TPA; 3, FDC-Mix 1 cells + plus Ca^{2+} ionophore; 4, FDC-Mix 1 cells + TPA+Ca^{2+} ionophore.

observed by Farrar *et al.* (1985), possibly due to the higher levels of protein kinase C that we found originally associated with the membrane fraction before IL-3 (or TPA) was added to the cells.

Activation of hexose transport by interleukin 3, TPA and Ca^{2+} ionophore

To analyse further this ability of TPA and Ca^{2+} ionophore to 'replace' interleukin 3 in FDC-Mix 1 cells, we have used one of the few known actions of IL-3 in the short

term, its ability to activate hexose transport. Previously we have shown that IL-3 can activate a D-hexose transport protein in FDC-P2 cells. We have suggested that this activation is central to survival of this IL-3-dependent cell line, in that increased glucose uptake promotes a higher rate of glycolytic flux and ATP production (Whetton *et al.* 1984) leading to survival and proliferation.

To confirm that the uptake and utilization of glucose is also important in IL-3-dependent FDC-Mix 1 cells, we have examined the effects of cytochalasin B (10 μM, a potent inhibitor of glucose transport *per se*) and 2-deoxyglucose (an inhibitor of glucose metabolism; Baroffia & Kucera, 1985; Michl *et al.* 1976) on FDC-Mix 1 cell survival and DNA synthesis stimulated by either IL-3 or TPA+Ca^{2+} ionophore. Both these agents markedly inhibit both survival and proliferation in FDC-Mix 1 cells (Fig. 4).

We have also examined the hexose transport system in FDC-Mix 1 cells. In all cells studied to date, D-hexose sugars only are taken up by a carrier-mediated process. We have found this to be true in FDC-Mix 1 cells; L-glucose is taken up by the cells at only 4 % of the rate of the D-hexose sugar 2-deoxyglucose. Furthermore, the D-hexose-transport protein inhibitor cytochalasin B (25 μM) inhibited 2-deoxyglucose uptake by FDC-Mix 1 by >95 %. Uptake of L-glucose is not inhibited by cytochalasin B. We have also found that IL-3 stimulates D-hexose transport in FDC-Mix 1 cells as it does in FDC-P2 cells and that this ability of IL-3 to activate hexose uptake is limited to D-sugars only. These results suggest that IL-3-mediated activation of D-hexose uptake is an important factor in the survival and proliferation events governed by IL-3 and that it can be used to investigate the role of TPA and Ca^{2+} ionophore in FDC-Mix 1 cell survival.

FDC-Mix 1 cells maintained in the absence of IL-3 for 4 h exhibit a hexose transport rate of 980 pmol min^{-1} per 10^6 cells using 2-deoxyglucose. Cells that are maintained in IL-3 during this 4-h period show a markedly higher (206 %) transport rate. Similarly, cells maintained in TPA, and Ca^{2+} ionophore + TPA, exhibit 2-

Table 2. *Effect of interleukin 3 and TPA on protein kinase C redistribution in FDC-Mix 1 cells*

Protein kinase C activity	^{32}P incorporated (cts min^{-1})
Membrane fraction	
No addition	652
+Interleukin 3 (100 units ml^{-1})	2852
+TPA (100 ng ml^{-1})	2418
Cytosolic fraction	
No addition	3516
+Interleukin 3 (100 units ml^{-1})	1670
+TPA (100 ng ml^{-1})	1652

Cells (10^7 ml^{-1}); 1 ml was incubated with the above additives for 10 min before cytosolic and membrane fractions were prepared as described by Farrar *et al.* (1985). Standard deviations for values above were <20 % on six observations.

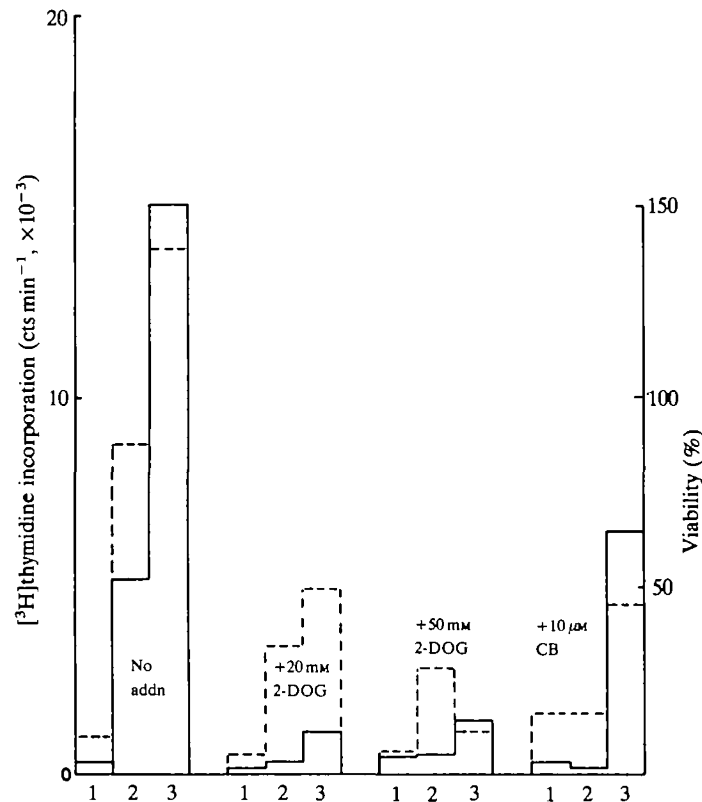

Fig. 4. Effect of cytochalasin B and 2-deoxyglucose on FDC-Mix 1 cell viability and DNA synthesis. The ability of cytochalasin B (CB) (10 μM) and 2-deoxyglucose (2-DOG, 20 and 50 mM) to inhibit IL-3-stimulated and TPA+Ca^{2+} ionophore-stimulated cellular survival and DNA synthesis was assessed after 24 h in the culture conditions shown above. Broken lines represent survival data; continuous lines represent [^{3}H]thymidine incorporation. 1. FDC-Mix 1 cells with no additives; 2, FDC-Mix 1 cells + TPA (100 ng ml^{-1}) and Ca^{2+} ionophore (100 ng ml^{-1}); 3, FDC-Mix 1 cells + IL-3 (100 units ml^{-1}).

deoxyglucose transport rates that are of the same order as cells maintained in IL-3. This suggests that these agents can act to maintain high transport levels over a 4-h culture period, while in the absence of any of these agents the transport rate falls to the value observed in control cultures. A corollary of this argument is that IL-3, TPA and Ca^{2+} ionophore should be able to restimulate hexose transport in FDC-Mix cells maintained in the absence of IL-3. This is shown to occur when IL-3, TPA, or TPA+Ca^{2+} ionophore, are added to the FDC-Mix cells (Table 3) but not on addition of Ca^{2+} ionophore alone. This suggests that TPA and IL-3 can stimulate and maintain hexose uptake in the short (4 min) and medium term (4·0 h), whereas Ca^{2+} ionophore can only increase transport rates in the medium term.

To assess any possible additivity or synergism between (optimal concentrations of) TPA, Ca^{2+} ionophore, TPA+Ca^{2+} ionophore, and high concentrations of IL-3 in the stimulation of hexose uptake, the effect of each of these additives was assessed

for its ability to activate FDC-Mix cells pretreated with TPA, Ca^{2+} ionophore, TPA+Ca^{2+} ionophore and IL-3 (see Table 3). In no case was additivity or synergism observed. Furthermore, short-term incubations with IL-3, IL-3+Ca^{2+} ionophore, TPA, or TPA+Ca^{2+} ionophore + IL-3, failed to show any additivity or synergism on hexose transport rates achieved by any of these agents at concentrations at which a maximal effect is achieved on DNA synthesis and survival when these agents are added alone (results not shown).

DISCUSSION

We have previously shown that one of the earliest events elicited by IL-3 is a stimulation of hexose uptake into target responder cells. The present work was undertaken to define the nature of the second messenger system(s) involved in this activation. Evidence presented in this paper and elsewhere (Farrar *et al.* 1985) demonstrates that, following binding of IL-3 to its receptor, there is a rapid

Table 3. *Effect of interleukin 3, TPA and Ca^{2+} ionophore on hexose uptake in FDC-Mix 1 cells*

Preincubation conditions	Incubation conditions	Hexose transport (% of control)
No addition	No addition	(100)
No addition	+Interleukin 3	185
No addition	+TPA	183
No addition	+Ca^{2+} ionophore	103
No addition	+TPA+Ca^{2+} ionophore	173
+Interleukin 3	No addition	206
+Interleukin 3	+Interleukin 3	189
+Interleukin 3	+TPA	174
+Interleukin 3	+Ca^{2+} ionophore	172
+Interleukin 3	+TPA+Ca^{2+} ionophore	171
+TPA	No addition	195
+TPA	+Interleukin 3	195
+TPA	+TPA	181
+TPA	+Ca^{2+} ionophore	165
+TPA	+TPA+Ca^{2+} ionophore	178
+Ca^{2+} ionophore	No addition	141
+Ca^{2+} ionophore	+Interleukin 3	179
+Ca^{2+} ionophore	+TPA	191
+Ca^{2+} ionophore	+TPA+Ca^{2+} ionophore	224
+TPA+Ca^{2+} ionophore	No addition	188
+TPA+Ca^{2+} ionophore	+Interleukin 3	196
+TPA+Ca^{2+} ionophore	+TPA+Ca^{2+} ionophore	189

Cells were prepared and incubated as described in Materials and Methods. Interleukin 3, TPA and Ca^{2+} ionophore (where used) were at concentrations of 10 units ml^{-1}, 100 ng ml^{-1} and 100 ng ml^{-1}, respectively. Control hexose transport rates using 2-deoxyglucose were 980 pmol min^{-1} per 10^6 cells. Results are the means of at least six experiments. Standard deviations were in all cases $<10\%$.

translocation of protein kinase C from the cytoplasm to the cell membrane. Because protein kinase C has been shown to be capable of phosphorylating the glucose transport protein (*in vivo* or *in vitro*) (Witters *et al.* 1985), we now propose that the ability of IL-3 to mediate survival and proliferation of primitive cells is associated with the activation of PK-C and the modulation (*via* phosphorylation) of the glucose transport protein. Obviously, this is speculative at present, but the validity of the model is strengthened by our experiments using TPA and Ca^{2+} ionophore. For example, the model predicts that at least *some* of the effect elicited by IL-3 should also be elicited by TPA, because in many other cell systems TPA has been shown to be a potent activator of PK-C (Nishizuka, 1984). Certainly, in our IL-3-dependent stem cell lines, TPA acts similarly to IL-3 in the translocation of PK-C to the cell membrane. Significantly, however, these initial events seen on exposure of the cells to TPA are followed by a range of biological responses that are very similar to those elicited by exposure of the cells to IL-3, suggesting some common mode of action between these two agents.

However, we can also observe a marked synergism between interleukin 3 + Ca^{2+} ionophore and TPA+Ca^{2+} ionophore in promoting survival and proliferation of FDC-Mix 1 cells, which implies that both the activation of protein kinase C and increases in cytosolic Ca^{2+} are important events in maintaining these cells. Furthermore, using the quin 2 Ca^{2+} indicator we have shown higher Ca^{2+} levels within IL-3-dependent cell lines maintained at high concentrations of growth factor compared with those at concentrations of IL-3 that only permit maintenance of the cells (A. D. Whetton, unpublished observations).

Increases in levels of cytosolic $Ca^{2+}$and activation of protein kinase C are both thought to be mediated by hormones and growth factors that stimulate phosphatidylinositol metabolism (Berridge & Irvine, 1984). The hydrolysis of phosphatidylinositol lipids to inositol trisphosphate and diacylglycerol leads to the increase in cytosolic Ca^{2+} and activation of protein kinase C, respectively. Ca^{2+} ionophore and TPA can also elicit these two events, and the synergistic interaction between these two agents has been shown to mimic the effects of hormones and growth factors that stimulate phosphatidylinositol catabolism in many biological systems (Kojima *et al.* 1983; Mastro & Smith, 1983; Rink *et al.* 1983; Tanaka *et al.* 1984). The evidence we have presented here suggests that IL-3 may promote the survival and proliferation of FDC-Mix 1 cells *via* its ability to stimulate phosphatidylinositol metabolism. We are examining this question now.

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REFERENCES

BAROFFIA, A. & KUCERA, P. (1985). The deoxyglucose method adapted for studies of glucose metabolism in the early embryo. *J. cell. Physiol.* **123**, 111–116.

BAZILL, G. W., HAYNES, M., GARLAND, J. & DEXTER, T. M. (1983). Characterization and partial purification of a haemopoietic cell growth factor in WEHI-3 cell conditioned medium. *Biochem. J.* **210**, 747–759.

BERRIDGE, M. J. & IRVINE, R. F. (1984). Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature, Lond.* **312**, 315–321.

BURGESS, A. W. & NICOLA, N. A. (1983). *Growth Factors and Stem Cells*, pp. 93–124. Australia: Academic Press.

DEXTER, T. M., BOETTIGER, D. & SPOONCER, E. (1985). Self renewal of haemopoietic stem cells: The roles of the environment, of growth factors, and of the *src* oncogene. *Haemat. Blood Transf.* **29**, 363–371.

DEXTER, T. M., GARLAND, J. M., SCOTT, D., SCOLNICK, E. & METCALF, D. (1980). Growth of factor dependent haemopoietic precursor cell lines. *J. exp. Med.* **152**, 1036–1047.

EAVES, A. C. & EAVES, C. J. (1984). Erythropoiesis in culture. *Clinics Haemat.* **13**, 371–391.

FARRAR, W. L., THOMAS, T. P. & ANDERSON, W. B. (1985). Altered cytosol/membrane enzyme redistribution on Interleukin 3 activation of protein kinase C. *Nature, Lond.* **315**, 235–237.

GOUGH, N. M., GOUGH, J., METCALF, D., KELSO, A., GRAIL, D., NICOLA, N. A., BURGESS, A. W. & DUNN, A. R. (1984). Molecular cloning of cDNA encoding a murine haematopoietic growth regulator, granulocyte–macrophage colony stimulating factor. *Nature, Lond.* **309**, 763–767.

KOJIMA, I., LIPPES, H., KOJIMA, K. & RASMUSSEN, H. (1983). Aldosterone secretion: effect of phorbol ester and A23187. *Biochem. biophys. Res. Commun.* **116**, 555–562.

MASTRO, A. M. & SMITH, M. C. (1983). Calcium dependent activation of lymphocytes by ionophore, A23187, and a phorbol ester tumor promoter. *J. cell. Physiol.* **116**, 51–56.

MICHL, J., OHLBAUM, D. J. & SILVERSTEIN, S. C. (1976). 2-Deoxyglucose selectively inhibits Sc and complement receptor mediated phagocytosis in mouse peritoneal macrophages. II. Dissociation of the inhibitory effects of 2-deoxyglucose on phagocytosis and ATP generation. *J. exp. Med.* **144**, 1484–1493.

NICOLA, N. A., METCALF, D., MATSUMOTO, M. & JOHNSON, G. R. (1983). Purification of a factor inducing differentiation in murine myelomonocytic leukaemia cells. Identification as a granulocyte colony stimulating factor. *J. biol. Chem.* **258**, 9017–9023.

NISHIZUKA, Y. (1984). The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature, Lond.* **308**, 693–698.

RINK, T. J., SANCHEZ, A. & HALLAM, T. J. (1983). Diacylglycerol and phorbol ester stimulate secretion without raising cytoplasmic free calcium in human platelets. *Nature, Lond.* **305**, 317–319.

SPOONCER, E., BOETTIGER, D. & DEXTER, T. M. (1984). Continuous *in vitro* generation of clonogenic multipotential stem cells from *src* infected cultures. *Nature, Lond.* **310**, 228–230.

STANLEY, E. R. & JUBINSKY, P. T. (1984). Factors affecting the growth and differentiation of haemopoietic cells in culture. *Clinics Haemat.* **13**, 329–348.

TANAKA, C., TANIYAMA, K. & KUSUNOKI, M. (1984). A phorbol ester and A23187 act synergistically to release acetylcholine from the guinea pig ileum. *FEBS Letts* **175**, 165–169.

WHETTON, A. D., BAZILL, G. W. & DEXTER, T. M. (1984). Haemopoietic cell growth factor mediates cell survival *via* its action on glucose transport. *EMBO J.* **3**, 409–413.

WHETTON, A. D. & DEXTER, T. M. (1983). Effect of haematopoietic cell growth factor on intracellular ATP levels. *Nature, Lond.* **303**, 629–631.

WITTERS, L. A., VATER, C. A. & LIENHARD, G. E. (1985). Phosphorylation of the glucose transporter *in vitro* and *in vivo* by protein kinase C. *Nature, Lond.* **315**, 777–779.

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