Okadaic acid mimics multiple changes in early protein phosphorylation and gene expression induced by tumor necrosis factor or interleukin-1*

(Received for publication, August 12, 1991)

G. R. Guy, X. Cao, S. P. Chua, and Y. H. Tan†
From the Institute of Molecular and Cell Biology, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511

Okadaic acid, a phosphatase inhibitor from a marine organism, mimics tumor necrosis factor/interleukin-1 (TNF/IL-1) in inducing changes in early cellular protein phosphorylation. A total of ~116 proteins exhibit significant and concordant changes in phosphorylation or dephosphorylation within 15 min in human fibroblasts activated by either okadaic acid, TNF, or IL-1. The fidelity of this mimicry by okadaic acid extends to the phosphorylation of the 27 hsp complex, stathmin, eIF-4E, myosin light chain, nucleolin, epidermal growth factor receptor, and other cdc2-kinase substrates (c-abl, RB, and p53). The okadaic acid-induced pattern of protein phosphorylation is distinct from that observed in cells treated with phorbol 12-myristate 13-acetate or with ligands like epidermal growth factor, cyclic AMP agonists, bradykinin, or interferons. Like TNF, okadaic acid also induces the transcription of immediate early response genes like c-jun and Egr-1 as well as the interleukin-6 genes. The overall early effects of okadaic acid uniquely parallel those of TNF/IL-1 and not those of other cytokines or ligands. Regulation of protein phosphatase inhibition is discussed as a mechanism for TNF/IL-1 signal transduction.

The control of the immune system is mediated through a complex interplay of lymphokines with different kinds of cells (1, 2). TNF and IL-1 are examples of lymphokines reported to be involved in the regulation of the host immune system (3–5). TNF and IL-1 are associated with disease states such as sepsis and autoimmune disorders and are described as mediators of the inflammatory response (4). Elevated levels of serum TNF in cancer, AIDS, and malaria are related to weight loss in these diseases (7). The association of these lymphokines with the disease state has prompted the application of TNF antibodies or inhibitors in the treatment of septic shock and the use of IL-1 antibodies in rheumatoid arthritis (8, 9). It also prompted us to search for natural products which could mimic or antagonize TNF/IL-1 activity and/or signal transduction in primary human cells.

IL-1 was originally described as a comitogen of thymocytes and TNF was first reported to be an endotoxin-induced serum factor which causes tumor necrosis (10, 11). The two lymphokines have similar biological activities on cells even though they do not have homologous amino acid sequences or homology in their receptors (12–15). Unlike the EGF, PDGF, and colony-stimulating factor-1 receptors which are known to have tyrosine kinase activity (16) and “serpentine receptors” that generate second messengers via GTP-binding proteins (17), little has been deduced of the mode of TNF or IL-1 signal transduction from their receptors. The involvement of GTP-binding proteins in TNF and IL-1 signalling processes was implied from several reports (18, 19). Increases in second messenger concentrations of cAMP, inositol 1,4,5-trisphosphate, and arachidonic acid have also been reported (20). The identification of several phosphorylated cellular substrates like the heat shock proteins, 1-plastin, stathmin, talin, and EGF receptor (EGFR) in TNF or IL-1 stimulated cells suggested the activation of serine/threonine kinase(s) (21, 22). In this connection, both lymphokines have been reported to concordantly induce early changes in the phosphorylation of 63 cytosolic proteins and to activate multiple protein kinases including microtubule-associated protein-2 and eIF-4E kinases in human fibroblasts (23).

TNF or IL-1 activates a number of transcription factors including NF-kB, NF-IL6, multiple response factor, interferon response factor-1, interferon response factor-2, c-fos, c-myc, c-jun, and NF-GH and induces the expression of several genes (24). Much has been described of TNF and IL-1 signalling effects at the transmembrane, cytosol, and gene expression levels, but the primary signalling pathway remains unresolved and in certain cases contradictory (21, 22, 25). We present data to show that okadaic acid, a phosphatase inhibitor derived from a marine black sponge (26), induces a composite pattern of early phosphorylation changes and gene expression in primary human fibroblasts strikingly in common with those induced by TNF or IL-1. This mimicry of signalling effects introduces another dimension to the mechanism of TNF/IL-1 signal transduction in which the regulation of protein phosphatase activity is shown to play a role.

**EXPERIMENTAL PROCEDURES**

Cell Cultures and Reagents—Human diploid fibroblasts, FS-4, were gifts from Dr. J. Vilcek (New York University). They were grown in tissue culture flasks and maintained with minimal essential medium supplemented with 10% fetal bovine serum (HyClone, Logan, UT) hereon referred to as regular medium. Human WISH cells were purchased from the American Type Culture Collection. Recombinant human TNF and IL-1 with specific activities of 2 x 10^10 units and 1 x 10^10 units/mg protein, respectively, and of purities of ≥90% were
purchased from Genzyme Corp. (Cambridge, MA). Phorboll2-myris-
phosphate, cells were plated onto 90-mm plastic tissue culture dishes. 
EGF were from Sigma. Okadaic acid was purchased from Biomol 
tate 13-acetate, bradykinin, forskolin, dibutyryl-cyclic AMP, and 
icotinate were washed twice with 5 ml of phosphate- and serum-free media (150 mM 
and incubated for 3 days for the cells to become confluent. Immedi-
ately before labeling, the media was removed and the cells were 
plate. The plates were incubated at 37 °C in a humidified CO2 incu-

Radiolabelling of Cells and Cell Lysis—For labeling with [32P]orthophosphate, cells were plated onto 90-mm plastic tissue culture dishes 
and incubated for 3 days for the cells to become confluent. Immedi-
ately before labeling, the media was removed and the cells were 
washed twice with 5 ml of phosphate- and serum-free media (150 mM 
and 0.5% glucose, 10 mM Tris, 0.1% bovine serum albumin, pH 7.4) before the addition of 1 
mCi/ml of [32P]orthophosphate in 3 ml of the same media to each 
plate. The plates were incubated at 37 °C in a humidified CO2 incu-

Two-dimensional Gel Electrophoresis—The hypophylized fractions (each of 12 µg) were subjected to isoelectric focusing for 18,000 V/h 
with pH 3–10 ampholytes using the Millipore Investigator two-
dimensional electrophoresis system, details of which are as previously 
described (23) (Millipore). Following this, the first-dimension gel was 
extracted and equilibrated in SDS buffer for 2 min before being 
loaded onto the second-dimension gel. The gel was fixed, dried, and 
and 32P-labeled poly peptides were located by autoradiography by using intensifying screens at -80 °C. For measure-

Protein Phosphatase in TNF/IL-1 Signal Transduction 

RESULTS 

Changes in Early Phosphorylation of Cellular Proteins in Cells Treated with Okadaic Acid, TNF, or Other Agonists—The 
32P-labeled protein spots are resolved and detected by autoradiography of the high definition two-dimensional gel 
electrophoresis of cytosolic extracts prepared from 32P-labeled human fibroblasts. The radioactive spots (each representing 
one or more phosphoproteins) are analyzed by computerized densitometry. Each spot is arbitrarily assigned a number. In 
addition, the intensity of each resolved spot derived from extracts of cells previously treated with an agonist is expressed 
relative to a basal level of phosphorylation in the absence of agonist. These changes in the level of phosphorylation are 
much faster than the changes in the level of phosphorylation in the absence of agonist. These changes are evident even at the basal level of phosphorylation in the absence of agonist. These changes in the level of phosphorylation are 
much faster than the changes in the level of phosphorylation in the absence of agonist. These changes are evident even at the 

Quantitative Determination of Protein Phosphorylation—The amount of protein phosphorylation on autoradiographs derived from 
cytosolic and nuclear/cytoskeletal-membrane fractions separated 
by one-dimensional gel electrophoresis combined with computerized imaging as previously described (23). Autoradiographs from control cytosol, 
TNF (50 units/ml)-treated cytosol (b), 200 nM OA-treated cytosol (c), 
untreated nuclear/cytoskeletal extract (d), TNF (50 units/ml) 
treated nuclear/cytoskeletal extract (e), and 200 nM OA-treated 
cytosol extract (f). Arrows indicate the prominent in-

Preparation of RNA and Northern Analysis—Human fibroblasts 
(4 x 10⁵) were seeded in 175-cm² tissue culture flasks and incubated 
at 37 °C for 6 days in regular medium. The cells were fed with regular 
medium on day 3. On day 6 the medium was replaced by minimal 
basal medium containing 0.25% fetal calf serum and incubated for 
another 48 h to maintain the cells in a quiescent state. Quiescent 
cells or cells maintained cells were treated with a specific agonist for 
the times indicated (see Fig. 7). The cells were harvested and total 
cytoplasmic RNA isolated by a one-step guanidinium thiocyanate/ 
phenol/chloroform method. RNA was subjected to electrophoresis 
through a 1.3% agarose gels containing 6.5% formaldehyde and then 
blotted to Hybond-N membranes. A BglII fragment of 1.6 kb corresponding to nucleotides 
302-1958 of Egr-1 cDNA was used as a probe. The hybridized 
cDNA probe was from the American Type Culture Collection (Rock-

to EGR-1 cDNA (23). The IL-6 

A typical example of the autoradiography of the two-dimensional gel analysis of the 
32P-labeled phosphoproteins derived from the cytosolic or 
nuclear/cytoskeletal membrane fractions prepared from cells 
treated with either TNF (50 units/ml) or okadaic acid (200 
nM) for 15 min is shown in Fig. 1. The prominent changes to 
protein phosphorylation in the ligand activated cells are indicated 
(arrow). In the cytosolic fraction, okadaic acid induced the same changes (85% identity) in early protein 
phosphorylation as TNF (Fig. 1, b and c). The phosphoryla-
tion pattern induced by IL-1 is not shown in Fig. 1 since the 
similarity between IL-1 and TNF has already been established 
(23). Okadaic acid also induced almost identical changes (as 
identified by computerized densitometry) in protein phos-
phorylation of the 46–50 proteins in the nuclear/cytoskeletal 
membrane fraction as did TNF treatment (Fig. 1, e and f). A summary of the changes in cytosolic protein phosphorylation is 
listed in Table I, providing a composite pattern of changes of 
protein phosphorylation in these fractions derived from 
human fibroblasts activated for 15 min by either TNF, oka-

FIG. 1. Comparison of agonist-induced changes in early 
protein phosphorylation in human fibroblasts. 32P-Labeled 
cultures were treated with TNF or okadaic acid (OA) for 15 min. The 
cells were then subjected to analysis by high resolution two-dimen-
sional gel electrophoresis combined with computerized imaging as 
previously described (23). Autoradiographs from control cytosol (a), 
TNF (50 units/ml)-treated cytosol (b), 200 nM OA-treated cytosol (c), 
untreated nuclear/cytoskeletal extract (d), TNF (50 units/ml) 
treated nuclear/cytoskeletal extract (e), and 200 nM OA-treated 
cytosol extract (f). Arrows indicate the prominent 

A
**Table I**

**Summary of pattern of changes in early protein phosphorylation in human fibroblasts**

Labeled fibroblasts were treated with either TNF (50 units/ml), 200 nM okadaic acid (OA), TPA (20 ng/ml), or combinations thereof as indicated for 15 min. Cytosolic and nuclear extracts were subjected to two-dimensional gel electrophoresis and the patterns of polypeptide phosphorylation on autoradiographs derived from cytosolic cell samples containing equal amounts of protein were analyzed by computerized densitometry as previously described (23). Results of five experiments are combined. Each radioactive spot was assigned a number (1-460) and a composite of phosphorylation changes identified from five separate experiments presented in the table. The changes in the level phosphorylation of each spot is matched against its untreated control as well as internal controls and scored. A positive score for each spot is recorded only when an increase is reproducibly observed in four out of five separate experiments. Scores of +, ++, and ++++, respectively, indicate a 100, 300-500, and 2500% increase in phosphorylation 15 min after agonist treatment. A minus (-) score indicates a 50% decrease in phosphorylation 15 min after agonist treatment.

| Spot number | TNF | OA | OA + TNF | TPA | TPA + TNF | TPA + OA |
|-------------|-----|----|----------|-----|-----------|----------|
| 4           | +   | ++ | ++       | +   | +         | +        |
| 10          | +   | +  | +        | +   | +         | +        |
| 12          | +   | +  | +        | +   | +         | +        |
| 13          | +   | +  | +        | +   | +         | +        |
| 19          | +   | +  | +        | +   | +         | +        |
| 24          | +   | +  | +        | +   | +         | +        |
| 34          | +   | +  | +        | +   | +         | +        |
| 46          | +   | +  | +        | +   | +         | +        |
| 51          | +   | +  | +        | +   | +         | +        |
| 57          | +   | +  | +        | +   | +         | +        |
| 58          | +   | +  | +        | +   | +         | +        |
| 60          | -   | -  | -        | -   | -         | -        |
| 61          | -   | -  | -        | -   | -         | -        |
| 62          | -   | -  | -        | -   | -         | -        |
| 63          | -   | -  | -        | -   | -         | -        |
| 64          | +   | +  | +        | +   | +         | +        |
| 65          | +   | +  | +        | +   | +         | +        |
| 66          | +   | +  | +        | +   | +         | +        |
| 78          | +   | +  | +        | +   | +         | +        |
| 80          | +   | +  | +        | +   | +         | +        |
| 81          | +   | +  | +        | +   | +         | +        |
| 82          | +   | +  | +        | +   | +         | +        |
| 106         | +   | +  | +        | +   | +         | +        |
| 107         | +   | +  | +        | +   | +         | +        |
| 108         | +   | +  | +        | +   | +         | +        |
| 109         | +   | +  | +        | +   | +         | +        |
| 110         | +   | +  | +        | +   | +         | +        |
| 111         | +   | +  | +        | +   | +         | +        |
| 116         | +   | +  | +        | +   | +         | +        |
| 117         | +   | +  | +        | +   | +         | +        |
| 134         | +   | +  | +        | +   | +         | +        |
| 139         | +   | +  | +        | +   | +         | +        |
| 140         | +   | +  | +        | +   | +         | +        |
| 148         | +   | +  | +        | +   | +         | +        |
| 153         | +   | +  | +        | +   | +         | +        |
| 156         | +   | +  | +        | +   | +         | +        |
| 161         | -   | -  | -        | -   | -         | -        |
| 162         | -   | -  | -        | -   | -         | -        |
| 163         | -   | -  | -        | -   | -         | -        |
| 164         | ++  | ++ | ++       | +   | +         | +        |
| 165         | +++ | +++| +++      | +++ | +++       | +++      |
| 166         | +   | +  | +        | +   | +         | +        |
| 167         | +   | +  | +        | +   | +         | +        |
| 168         | +   | +  | +        | +   | +         | +        |
| 169         | +   | +  | +        | +   | +         | +        |
| 171         | +   | +  | +        | +   | +         | +        |
| 172         | +++ | +++| +++      | +++ | +++       | +++      |
| 173         | +   | +  | +        | +   | +         | +        |
| 174         | +   | +  | +        | +   | +         | +        |
| 175         | +   | +  | +        | +   | +         | +        |
| 176         | +   | +  | +        | +   | +         | +        |
| 179         | +   | +  | +        | +   | +         | +        |
| 180         | +   | +  | +        | +   | +         | +        |
| 184         | +   | +  | +        | +   | +         | +        |
| 185         | +   | +  | +        | +   | +         | +        |
| 187         | +   | +  | +        | +   | +         | +        |

Okadaic acid, TPA, or combinations of these ligands. In the numerous experiments performed okadaic acid is shown to mimic TNF in inducing changes in the early phosphorylation of ~60 and the dephosphorylation of six proteins in the cytosolic fraction during the first 15 min of ligand treatment (Table I). Combinations of optimal doses of okadaic acid (200 nm) and TNF (50 units/ml) in the treatment of fibroblasts do not produce an additive or synergistic effect on these.
changes (Table I). TPA (an activator of protein kinase C) treatment of human fibroblasts produced a pattern of phosphorylation changes quite different from that produced by okadaic acid or TNF (Table I). TPA in combination with either okadaic acid or TNF produced a pattern of phosphorylation changes which represents the sum of complementary changes produced by each agonist (Table I). These results indicate that okadaic acid and TNF produce early changes in protein phosphorylation through a common pathway which is different from that of TPA-induced activation of protein kinase C. Similarly, the pattern of protein phosphorylation induced in cells treated by bradykinin, cAMP agonists, EGF, or IFN-\(\alpha\) is different from that induced by okadaic acid or by TNF/IL-1 (data not shown). Furthermore, treatment of human fibroblasts by IFN-\(\gamma\) did not produce measurable changes in early protein phosphorylation.

A number of the ~66 proteins in the cytosol fraction and the 46–50 proteins in the nuclear/cytoskeletal membrane fraction which undergo the early phosphorylation changes have now been identified by either using marker proteins or by their molecular weights and isoelectric points. They include the 27 hsp complex (identified as 165, 172, and 176 in Table I), stathmin (as S [27] in Fig. 1, b and c), nucleolin (as N [28] in Fig. 1, e and f), and myosin light chain (MLC in Fig. 1, e and f). The phosphorylation of these proteins is concordantly enhanced in cells activated by TNF (Fig. 1, b and e), by okadaic acid (Fig. 1, c and f) or by IL-1 (not shown).

In a separate experiment using m'GTP-Sepharose to isolate the eIF-4E from okadaic acid-treated human fibroblasts by procedures previously reported (23) for TNF and IL-1, okadaic acid also enhanced the phosphorylation of this protein after 15 min by 2–3-fold (Fig. 2). Okadaic acid treatment of cells was extended to WISH cells and again produced almost identical changes in early protein phosphorylation as TNF (not shown), indicating that the mimicry of the early events of TNF signal transduction by okadaic acid is not confined to primary human fibroblasts.

It is important to note that though the TNF or IL-1 induced changes in protein phosphorylation shown in Fig. 1 and Table I were measured 15 min after ligand treatment, these changes were detectable within 3 min and maximal at ~15 min before returning to basal levels at 45–60 min. By then most of the changes in phosphorylation induced by TNF or IL-1 are no longer measurable.

**Dose-response and Time Course of Okadaic Acid-induced Changes in the Phosphorylation of the 27 hsp Complex**—The 27 hsp complex is most sensitive to phosphorylation changes induced by okadaic acid and TNF (Fig. 1). The concentration-dependent effect of okadaic acid on changes in phosphorylation of the 27 hsp complex was measured and compared with those in cells treated with 50 units/ml of TNF (Fig. 3). It is apparent from this comparison that treatment of cells with 50 units/ml of TNF for 15 min produces about the same increase in the phosphorylation of the 27 hsp complex as treatment of cells with between 160–320 nM okadaic acid for 15 min (Fig. 3, c-e).

A measurement of protein phosphorylation of the 27 hsp complex was performed in cells treated with 200 nM okadaic acid for 0, 5, 15, and 30 min. This measurement shows proportional increases in phosphorylation of the 27 hsp complex with increasing time of okadaic acid treatment (Fig. 4, a–d). After 30 min of okadaic acid treatment the 27 hsp complex was hyperphosphorylated.

**IL-1, TNF, or Okadaic Acid Treatment Stimulate Phosphorylation of the EGFR Receptor**—One feature of TNF and IL-1 action is the transmodulation of the EGFR via phospho-

---

**Figure 2.** Phosphorylation of cap-binding protein by cytosolic extracts of human fibroblasts treated with TNF (50 units/ml), IL-1 (50 units/ml), or OA (okadaic acid) at the indicated concentration for 15 min. The \(^{32}\)P-labeled cap-binding protein (27,000 kDa) as purified by SDS-PAGE was quantified by computerized densitometry and compared in relative phosphorylation intensity.

**Figure 3.** Dose-dependent phosphorylation of the 27 hsp complex by okadaic acid or TNF. Human fibroblasts prelabeled with \(^{32}\)Porthophosphate (1 mCi/ml) were incubated with increasing concentrations of OA or TNF. Cytosolic fractions were prepared from the cells and subjected to two-dimensional gel electrophoresis. a, control; b, 80 nM OA; c, 160 nM OA; d, 50 units/ml TNF; and e, 320 nM OA.

**Figure 4.** Time-course of phosphorylation of the 27 hsp complex of the cytosolic fraction of human fibroblasts prelabeled with \(^{32}\)Porthophosphate (1 mCi/ml) by okadaic acid. The fibroblasts were treated with 200 nM of okadaic acid for 0 min (a), 5 min (b), 15 min (c), or 30 min (d). Cytosolic fractions were prepared from the fibroblasts and analyzed by two-dimensional gel electrophoresis.
phorylation (29, 30). Okadaic acid was added to human fibroblasts to see if it can also mimic this effect of TNF and IL-1.

**Phosphorylation of okadaic acid or TNF or IL-1, 60% of the changes were in the cytosolic fraction and 40% in the nuclear and cytoskeletal/...**

Both proteins are known to be substrates of cdc2 kinase. In this connection, a question was raised as to whether other cdc2 kinase substrates which are known to be phosphorylated by okadaic acid treatment (31, 32) are also affected in TNF-treated human fibroblasts. Three substrates of cdc2 kinase, namely c-abl, RB, and p53, were tested. Like okadaic acid, TNF induced the phosphorylation of all three cdc2 kinase substrates in human fibroblasts. Okadaic acid or TNF enhanced the phosphorylation of c-abl, RB, and p53 (Fig. 6). The results also show that the RB proteins are found in two forms, one having slightly higher molecular mass (115 kDa) than the other (110 kDa). The 115-kDa protein is likely the hyperphosphorylated RB protein, whereas the 110-kDa protein represents the hypophosphorylated form (33). Like okadaic acid treatment of cells, TNF increases the ratio of the hyperphosphorylated RB protein to the hypophosphorylated form of RB (Fig. 6). Similarly the p53 protein is present in two very closely associated forms (34), the slightly higher molecular mass species being more heavily phosphorylated than the slightly lower molecular mass species. Like TNF-treated cells, okadaic acid-treated fibroblasts showed 3- to $\geq 5$-fold higher amounts of the hyperphosphorylated RB and p53 protein than the lesser phosphorylated forms of these proteins.

**Induction of Egr-1, c-jun, and IL-6 Genes by TNF and Okadaic Acid—**Having shown the early changes in protein phosphorylation in the cytosol and nuclear/cytoskeletal membrane fractions of okadaic acid-, TNF-, and IL-1-treated fibroblasts, we investigated the effects of okadaic acid on gene expression. TNF is known to induce eight genes in primary human fibroblasts (35). Recently we observed the induction of an immediate early response gene, the Egr-1 gene, in primary human fibroblasts in response to TNF. The induction of expression of three TNF/IL-1-inducible genes by okadaic acid was preliminarily examined in primary human fibroblasts. The three genes selected were Egr-1, c-jun (36) and IL-6 (37). Like TNF, okadaic acid effectively induced all these genes. Egr-1 was rapidly induced within 30 min and c-jun within 60 min of okadaic acid treatment, whereas IL-6 was induced much later at 7 h after okadaic acid treatment (Fig. 7). We have no explanation concerning the late induction of IL-6 by okadaic acid except to note that similar late induction of c-fos by okadaic acid was recently reported as well (43).

**DISCUSSION**

In this study, we found that okadaic acid induces changes in early protein phosphorylation in primary human fibroblasts similar to that induced by TNF or IL-1 (Fig. 1 and Table 1). Out of the 116 proteins affected by treatment of cells with okadaic acid or TNF or IL-1, 60% of the changes were in the cytosolic fraction and 40% in the nuclear and cytoskeletal/...
membrane fractions (Fig. 1). A number of these phosphoproteins were identified, including the EGF, 27 hsp complex, stat5bmin, c-SFK, 4E, myosin light chain, nucleolin, and cdc-2 kinase substrates such as c-abl, RB, and P53 proteins (Figs. 1, 2, 5, and 6). Like TNF, okadaic acid also induces the transcription of Egr-1, c-jun, and IL-6 genes in human fibroblasts (Fig. 7). However, unlike TNF or IL-1 the protein phosphorylation induced by okadaic acid increased proportionally with the time of treatment (Fig. 4). No subsequent decrease was observed during the first 2 h of treatment. Longer treatment (30 min or more) resulted in the hyperphosphorylation of proteins. In this respect, the changes induced by okadaic acid differ from those observed upon TNF/IL-1 treatment which produces transient increases in cellular protein phosphorylation. An explanation for this difference is that IL-1 and TNF receptors are known to be internalized upon ligand activation and hence their effects can be down-regulated with time (23). On the other hand, okadaic acid accumulates in the treated cells and continues to exert its effect on the phosphorylation of the target proteins resulting in the hyperphosphorylation of these target proteins in the cell. Other than this, the fidelity of mimicry of the early events of TNF or IL-1 action by okadaic acid is maintained from the levels of cytosolic and nuclear protein phosphorylation to that of gene induction (Fig. 1 and Fig. 7). In comparison with other ligands [namely TPA (Table I), cAMP agonists, EGF, bradykinin, or IFN (not shown)], TNF, IL-1, or okadaic acid induce a distinct pattern of cellular protein phosphorylation. In addition even though okadaic acid can mimic a few aspects of EGF action, such as the transmodulation of the EGFR phosphorylation (Fig. 5), it is evident from the comparison of the early changes in protein phosphorylation and gene induction that overall okadaic acid effects are uniquely parallel to those of TNF/IL-1 and not to those of other cytokines or ligands tested.

The striking similarity of changes in the phosphorylation of cellular proteins induced by okadaic acid and TNF reported herein indicates that when human fibroblasts are treated with either of the agonists, similar signal transduction pathways are initiated. In this regard, the inhibitory effect of okadaic acid on protein phosphatase-2A and to a lesser extent on protein phosphatase-1 (38) provides clues to a possible mechanism of its mimicry of TNF/IL-1 actions as well as to the mechanism of TNF/IL-1 signalling itself. A number of explanations are discussed. It is possible that the phosphorylation state of the substrates of TNF/IL-1-activated protein kinase(s) is tightly regulated by an okadaic acid-sensitive phosphatase(s). The inactivation of the phosphatase(s) by okadaic acid would alter the balance of the opposing phosphorylation/dephosphorylation in favor of net phosphorylation. Another explanation is that TNF/IL-1 treatment leads to the inactivation of an okadaic acid-sensitive protein phosphatase(s). In this scenario the inhibition of dephosphorylation will, as above, result in an overall increase in the phosphorylation of the cellular protein substrates by the opposing protein kinase.

This explanation would account for the relatively large number of proteins showing net increases in phosphorylation after cells are treated with TNF/IL-1 or okadaic acid. It is also possible that okadaic acid treatment can indirectly activate the same protein kinases that TNF/IL-1 treatment activates. The indirect activation of these kinases can occur via the inhibition of a kinase suppressor, a suppressor which is active in the dephosphorylated form. In this connection, okadaic acid has been shown to possibly activatecdc2 and microtubule-associated protein-2 kinase through this mechanism (39–41). The inactivation of a protein phosphatase as a signalling mechanism is unprecedented. There are several examples of specific protein phosphorylations that are regulated at the level of protein kinase(s) and phosphatase(s) (42). The present finding of the mimicry of TNF/IL-1 by okadaic acid introduces the concept of protein phosphatase regulation as one important mechanism by which the signal transduction of TNF/IL-1 is mediated.

Acknowledgments—We thank Dr Catherine J. Pallen for critical review and reading of the manuscript and Teresa Seow for typing.

REFERENCES

1. Balkwill, F. R., and Burke, F. (1989) Immunol. Today 10, 293–294.
2. Mantovani, A., and Dejana, E. (1989) Immunol. Today 10, 375–380.
3. Paul, N. L., and Ruddle, N. H. (1988) Annu. Rev. Immunol. 6, 407–438.
4. Dinarello, C. A. (1990) in Lymphokines and the Immune Response (Cohen, S., ed) pp. 146–179, CRC Press, Boca Raton, Fl.
5. Buetler, B., and Cerami, A. (1989) Annu. Rev. Immunol. 7, 625–655.
6. Dinarello, C. A. (1984) Trends Pharmacol. Sci. 5, 420.
7. Goh, C. R. (1990) Ann. Acad. Med. 19, 235–239.
8. Wu, W. Y., Kasperud, J. A., Thompson, R. C., and Dinarello C. A. (1991) FASEB J. 5, 338–343.
9. Tracey, K. J., Fong, Y. M., and Hesse, D. G. (1987) Nature 330, 662–664.
10. Gery, I., and Waksman, B. H. (1972) J. Exp. Med. 136, 143–155.
11. Carswell, E. A., Old, L. J., Kassel, R. L., Greene, S., Fiore, N., and Williamson B. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3666–3670.
12. Loetsher, H., Pan, Y.-C. E., Lahm, H. W., Gentz, R., Brockhaus, M., Tabuchi, H., and Lessauer, W. (1990) Cell 61, 351–359.
13. Schall, T. J., Lewis, M., Koller, K. J., Lee, A., Rice, G. C., Wong, G. H. W., Gatanaga, T., Granger, G. A., Lentz, R., Raab, H., Kohr, W. J., and Goeddel D. V. (1990) Cell 61, 361–370.
14. Sims, J. E., March, C. J., Cosman, D., Widmer, M. B., MacDonald H. R., MacMahan C. J., Grubin, C. E., Wignall J. M., Jackson J. L., Call, S. M., Friend, D., Alpert, A. R., Gillis, S., Urdai, D. L., Dower, S. K. (1988) Science 241, 585–589.
15. Smith, C. A., Davis, T., Anderson D., Solam, L., Beckmann, M. P., Jerzy R., Dower, S. K., Cosman, D., and Goodwin, R. G. (1990) Science 248, 1019–1023.
16. Ullrich, A., and Schlessinger, J. (1990) Cell 61, 203–212.
17. Birrbaumer, L., Abramowitz, J., and Brown, A. M. (1990) Biochim. Biophys. Acta 1031, 163–224.
18. O’Neill, L. A. J., Bird, T. A., Gearing, A. J. H., and Saklatvala, J. (1990) J. Biol. Chem. 265, 3146–3152.
19. Imamura, K., Sherman, K. L., Spriggs, D., and Kufe, D. (1989) J. Biol. Chem. 263, 10247–10253.
20. Guy, G. R., Ng, S. B., and Chua, S. P. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 6497–6500.
21. Qwarnstrom, E. E., MacFarlane, S. A., Page, R. C., and Dower, S. K. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 1232–1236.
22. O’Neill, L. A. J., Bird, T. A., and Saklatvala, J. (1990) Immunol. Today 11, 392–394.
23. Guy, G. R., Chua, S. P., Wong, N. S., Ng, S. B., and Tan, Y. H. (1991) J. Biol. Chem. 266, 14343–14352.
24. Vilcek, J., and Lee, T. H. (1991) J. Biol. Chem. 266, 7313–7316.
25. Mizei, S. B. (1990) Immunol. Today 11, 390–391.
26. Tachibana, K., Scheuer, P. J., Tsukitani, Y., Kikuchi, H., Van Engen, D., Claridy, J., Gopichand, Y., and Schmitz, F. J. (1981) J. Am. Chem. Soc. 103, 2469–2471.
27. Pagaraas, M. O., Bishop, J. F., Rinaudo, M. S., and Axelrod, J. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 2555–2559.
28. Issinger, O.-G., Martin, T., Richter, W., Olson, M., and Fujiki, H. (1988) EMBO J. 7, 1621–1626.
29. Donato, N. J., Gallic, G. E., Steck, P. A., and Rosenblum, M. G. (1989) J. Biol. Chem. 264, 20474–20481.
30. Bird, T. A., and Saklatvala, J. (1988) J. Immunol. 142, 126–133.
31. Luscher, B., Bruzulenco, L., Beach, D., and Eisenman, R. N. (1991) J. Immunol. 146, 2692–2697.
32. Kipreos, E. T., and Wang, J. Y. J. (1990) Science 248, 217–220.
33. Thomas, N. S. B., Burke, L. C., Bybee, A., and Linch, D. C. (1991) Oncogene 6, 317–322.
Protein Phosphatase in TNF/IL-1 Signal Transduction

34. Bischoff, J. R., Friedman, P. N., Marshak, D. R., Prives, C., and Beach, D. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4766-4770
35. Lee, T. H., Lee, G. W., Ziff, E. B., and Vilcek, J. (1990) Mol. Cell. Biol. 10, 1982-1988
36. Brenner, D. A., O'Hara, M., Angel, P., Chojkier, M., and Karin, M. (1989) Nature 337, 651-653
37. Ray, A., Tatter, S. B., May, L. T., and Sehgal, P. B. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6701-6705
38. Bialojan, C., and Takai, A. (1988) Biochem. J. 258, 283-290
39. Yamashita, K., Yasuda, H., Pines, J., Yasumoto, K., Nishitani, H., Ohtsubo, M., Hunter, T., Sugimura, T., and Nishimoto, T. (1990) EMBO J. 9, 4331-4338
40. Gotoh, Y., Nishida, E., and Sakai, H. (1990) Eur. J. Biochem. 193, 671-674
41. Felix, M.-A., Cohen, P., and Karsenti, E. (1990) EMBO J. 9, 675-683
42. Cohen, P. (1989) Annu. Rev. Biochem. 58, 453-508
43. Schonthal, A., Tsukitani, Y., and Feramisco, J. R. (1991) Oncogene 6, 423-430