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Effects of Tumor Necrosis Factor α (TNFα) on the Phospholipid Metabolism of *Tetrahymena pyriformis*

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The effect of (0·05 ng ml^{-1} and 0·1 ng ml^{-1}) TNFα on the phospholipid metabolism of *Tetrahymena pyriformis* was studied. The amount of phosphatidyl choline (PC), phosphatidyl inositol (PI), phosphatidic acid (PA), phosphatidyl ethanolamine (PE), diacylglycerol (DAG), arachidonic acid (AA) and ceramide was higher, but the phosphatidyl inositol 4 phosphate (PIP) and phosphatidyl inositol bis-phosphate (PIP_2) as well, as sphingomyelin (SM) content was lower in TNFα-treated cells than in the controls. In the culture medium (secreted forms) this situation was reversed. There were differences in the results gained by incorporation of [^{3}H]-palmitic acid or ^{32}P into the phospholipids. To control the functional effects of TNFα in *Tetrahymena*, the rate of cell division, the condensation of chromatin, the viability of cells and morphometrical values have been studied. The cytokine reduced cell growth, altered morphometric indices and increased chromatin condensation, however cell viability was not influenced. The results demonstrate the effects of TNFα at a low level of evolution, what is realized by changes in the phospolipid metabolism participating in signalling pathways. © 1998 John Wiley & Sons, Ltd.

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KEY WORDS — phospholipids; ceramide; sphingomyelin; chromatin condensation; TNFα; *Tetrahymena*

INTRODUCTION

The cytokine tumor necrosis factor α (TNFα) has multiple biological activities. It is one of the most potent physiological inducers of the nuclear transcription factor NFκB:[1] it induces the stress-activated protein kinases (SAPKs, also known as Jun nuclear kinases) that results in the stimulation of AP-1-dependent gene transcription and induces the translocation of NFκB to the nucleus provoking the stimulation of NFκB-dependent gene transcription. Most of the inflammatory and proviral effects of TNFα are mediated through the activation of the NFκB. A potential second messenger for these signalling pathways is ceramide, which is generated when TNFα activates sphingomyelinases.[2]

In many tumor cell lines, TNFα causes apoptosis with characteristic internucleosomal fragmentation and chromatin condensation; in this phenomenon the TNFα-generated ceramide derived from hydrolysis of the membrane sphingomyelin also plays a very important role.[3]

TNFα causes a rapid increase of diacylglycerol (DAG) via hydrolysis of phosphatidylcholine (PC) by PC-specific phospholipase C (PC-PLC).[4] The DAG generated by the TNFα-responsive PC-PLC leads to the activation of acidic sphingomyelinase (Smase) which causes breakdown of sphingomyelin to produce ceramides. The action of DAG on Smase is independent of its effects on protein kinase C (PKC); the PKC-activating phorbol esters do not alter Smase activity.[5]

TNFα shows diverse effects on the lipid metabolism of different cell types. Arachidonic acid (AA) release by TNFα occurs in a number of cell lines; TNFα increases the activity of cytosolic phospholipase A_2 ($cPLA_2$), the enzyme which is specific for the release of AA from the sn-2 position of membrane phospholipids.[6] TNFα-triggered release of AA provides a source for the production of eicosanoids. Pretreatment of human neutrophils

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with TNFα causes a rapid increase of the incorporation of AA into phosphatidylinositol (PI) and phosphatidic acid (PA) and a slower rise in the incorporation into PC and phosphatidylethanolamine (PE). TNFα in human neutrophils decreased — through the release of AA — the amount of PI, PC and PE.[7] In Caco 2 intestinal cells significant inhibition of lipid secretion by TNFα was observed,[8] on the other hand, TNFα stimulated phospholipid secretion in Hep G_2 human hepatoma cell line.[9] In pneumocytes TNFα resulted in a decreased PC synthesis; this inhibitory effect was completely blocked by indomethacin, which indicates that the effect of TNFα is mediated by prostaglandins in these cells.[10]

The unicellular *Tetrahymena pyriformis* has receptors that bind hormones of vertebrates,[11–13] synthesizes hormones similar to mammalian ones[14] and utilizes many signalling pathways analogous to those of mammalian cells, including the phosphatidylinositol[15] and glycosyl phosphatidylinositol (GPI)[16] systems. Enzymes catalysing the turnover of these lipid second messengers (phospholipase A_2;[17] phospholipase D{PLD}[18]) were also demonstrated. Suitable inhibitors or stimulators alter the turnover of these systems in *Tetrahymena*. For example insulin treatment enormously increases the activity of PLD,[19] neomycin prevents the synthesis of GPI and the turnover of the PI system,[20] the PKC-activating phorbol esters increase ^{32}P incorporation into the inositol phospholipids.[21]

Our major objective in this study, was to characterize the effect of TNFα on the phospholipid turnover of *Tetrahymena*. In an attempt to understand the effects of TNFα on *Tetrahymena* we have also studied the growth capacity, viability and the chromatin condensation of *Tetrahymena* cells in the presence of TNFα, and the effect of this cytokine on the morphological features of the unicellular organism.

MATERIALS AND METHODS

Materials

Human recombinant TNFα and lipid chromatographic standards (PI, PIP, PIP_2, PE, PA, PC, sphingomyelin, ceramide) were obtained from Sigma (St. Louis, MO, U.S.A.). [^{32}P]-Na orthophosphate (specific activity 7 Gbq mM^{-1}) was purchased from Izinta (Budapest, Hungary). [9, 10-^{3}H] Palmitic acid (specific activity 2·0 TBq mM^{-1}) was obtained from Amersham (Buckinghamshire, U.K.). Silica gel G chromatography plates were obtained from Merck (Darmstadt, Germany). Tryptone and yeast extract were obtained from Difco (Michigan, U.S.A.). All other chemicals used were of analytical grade available from commercial sources.

Tetrahymena *Cultures*

In the experiments *Tetrahymena pyriformis* GL strain was tested in the logarithmic phase of growth. The cells were cultivated at 28°C in 0·1 per cent yeast extract containing 1 per cent tryptone medium. Before the experiments the cells were washed with fresh culture medium, and were resuspended at a concentration of 5×10^6 cells ml^{-1}.

Effect of TNFα on the Metabolism and Secretion of [^{3}H]-Palmitic Acid Labelled Phospholipds of Tetrahymena

Tetrahymena cultures were treated with 1·5 μCi ml^{-1} [^{3}H]-palmitic acid for 2 h. After incubation the cells were washed thrice with fresh culture medium. The labelled cell population was placed into six glass centrifuge tubes (2 ml; 5×10^6 cells ml^{-1}): two tubes served as controls, two tubes were treated with 0·05 ng ml^{-1} TNFα and another two tubes with 0·1 ng ml^{-1} TNFα. The incubation was stopped by rapid chilling of the cells on ice, the mixture was centrifuged, to separate the cell pellet and supernatant after 10 and 60 min.

The lipid content from the cell pellet was separated according to the method of Bligh and Dyer.[22] From the supernatant (~2 ml) the lipids were separated by adding 4 ml chloroform: methanol (1:2 v/v) and 1 ml water. The chloroform phase was separated and dried under a stream of N_2.

The lipids were separated on silica gel chromatography plates with chloroform:methanol:water (65:35:2·5 v/v/v) solvent system. After development, 0·5 cm strips were scraped from the chromatograms into scintillation vials, and the radioactivity was measured with a Beckman scintillation counter. The individual lipids were identified by a parallel run of authentic standards.

Effect of TNFα on the Metabolism and Secretion of ^{32}P-Labelled Phospholipids of Tetrahymena

Tetrahymena cultures were treated with 9 μCi ml^{-1} [^{32}P]-Na orthophosphate for 1 h. The treatments of cells and the separation of lipids were

done by the method described above. The chromatograms were covered with Kodak TMG X-ray film. After exposure (~18 h) and development of radiograms, the radioactivity of individual phospholipid spots was analysed by laser densitometer (Ultroscan XL, Pharmacia-LKB, Uppsala, Sweden). The individual phospholipids were expressed as a percentage of the total incorporated ^{32}P which was taken as 100 per cent. The phospholipids were identified by a parallel run of authentic phospholipid standards.

Effect of TNFα on the Incorporation of ^{32}P into the Phospholipids of Tetrahymena

Tetrahymena cells were washed and resuspended in fresh culture medium and were divided into three groups (30 ml; 5×10^6 cells ml^{-1}): (a) control group; (b) 0·05 ng ml^{-1} TNFα-treated group and (c) 0·1 ng ml^{-1} TNFα-treated group. After 30 min 18 Mbq [^{32}P]-Na orthophosphate was added to each experimental group. Samples of 5 ml were taken after 1, 5, 15, 30 and 60 min. The phospholipids were separated by the method of Suchard *et al.*[23] The phospholipid samples were analysed on silica gel chromatography plates using the solvent system, chloroform:acetone:methanol:acetic acid:water (40:15:13:12:8 v/v). The phospholipids were identified and quantified by the methods described above.

Effect of TNFα on the Growth Rate of Tetrahymena pyriformis *Populations*

Tetrahymena cultures were washed and resuspended in fresh culture medium to a final cell density of 5×10^4 cells ml^{-1}, and this cell population was divided into two experimental groups (in 10 ml): (a) control group; (b) 0·1 ng ml^{-1} TNFα-treated group. Cells were counted in fixed samples (0·5 ml) using a Fuchs–Rosenthal counting chamber at 0, 4, 18 and 28 h of cultivation.

The viability of cells was examined by trypan blue exclusion.

Effect of TNFα on the Chromatin Condensation of Tetrahymena

Washed and resuspended *Tetrahymena* cultures in fresh medium (5×10^6 cells ml^{-1}) were divided into three experimental groups: (a) control group; (b) 0·05 ng ml^{-1} TNFα-treated group and (c) 0·1 ng ml^{-1} TNFα treated group. Samples were taken after 10 and 60 min. The cells were fixed in 4 per cent formalin solution (in pH 7·2 PBS), washed in two changes of PBS and Feulgen reaction for DNA was done.[24] The duration of hydrolysis with 1 M HCl at 60°C was 30 min.

The colour intensity of the Feulgen reaction (transparence, ϑ) was assessed with a Zeiss Amplival cytophotometer at 546 nm. One hundred cells were evaluated in each group.

Morphometric Analysis of the Tetrahymena *Cells*

The treatment of the cells was by the method described in the case of chromatin condensation. Prior to the analysis, the samples were stained with 0·1 per cent toluidine blue for 2 min. The gently washed cells were applied to a microscopic slide and were photographed under a light microscope (magnification: 800×). The prints were scanned with a Hewlett Packard HP-Scan Jet II scanner and the data obtained were analysed by a computer program (Biomorph 1.1). The interactive morphometry program allowed us to determine the area and the ratio of the shortest and longest axis (W/L) of the cells.

Statistical Treatment of the Data

In each experiment, the experimental data represent the means of quadruplicate experiments. Student's *t*-test was used for calculations, with $p < 0{\cdot}05$ accepted as the lowest level of statistical significance.

RESULTS

Effect of TNFα on the Metabolism and Secretion of [^3H]-Palmitic Acid Labelled Phospholipids of Tetrahymena

In the presence of TNFα the metabolism and secretion of [^{3}H]-palmitic acid labelled phospholipids after 60 min showed marked alteration — compared with the controls. Shorter treatments (10 min) resulted in no significant alterations in these parameters. The PC, PI, PA and PE content of the cells was higher than in the controls, and also the part of chromatogram ($Rf = 0{\cdot}9$–$0{\cdot}95$) — where DAG, AA and ceramide appeared — showed higher radioactivity than the controls; however the PIP, PIP_2 and SM-containing region of chromatograms contained lower incorporated

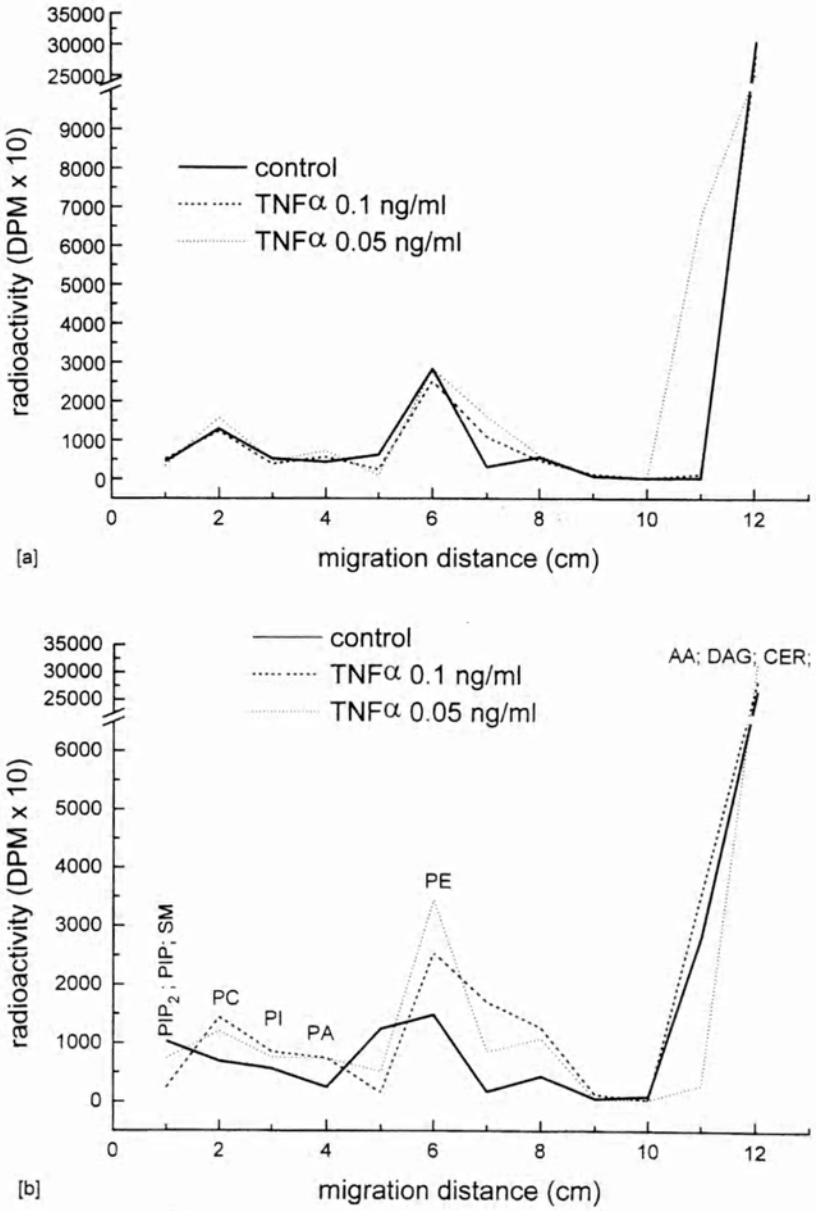

Figure 1. Effect of TNFα on the [^{3}H]-palmitic acid labelled phospholipid content of *Tetrahymena pyriformis*. Incubation with [^{3}H]-palmitic acid was for 60 min, and the washed cells were inoculated into fresh culture medium or TNFα-containing mediums. Lipid extraction from the cells after 10 min (a) and 60 min (b). The experiments were done four times with a representative experiment shown.

[^{3}H]-palmitic acid content than the controls (Figure 1).

In the culture medium the opposite situation was found: the amount of secreted phospholipids and DAG was significantly lower in the case of TNFα-treated groups than in the controls (Figure 2).

Effect of TNFα on the Metabolism and Secretion of ^{32}P Labelled Phospholipids of Tetrahymena

In the case of ^{32}P labelling, certain differences were detected in the metabolism and secretion of phospholipids, compared with the [^{3}H]-palmitic

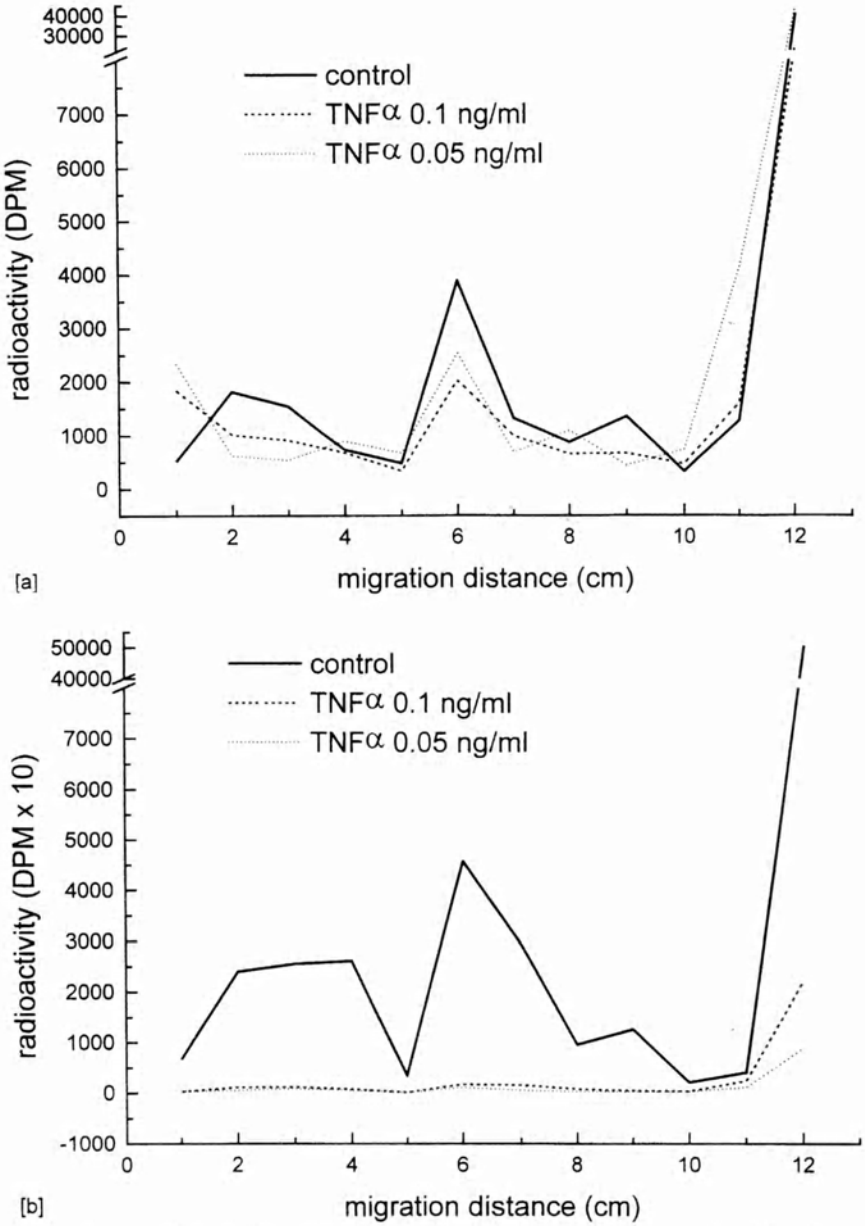

Figure 2. Effect of TNFα on the [^{3}H]-palmitic acid labelled phospholipid content of culture media of *Tetrahymena pyriformis*. The treatments as in Figure 1. Lipid extraction from the culture media after 10 min (a) and 60 min (b). The experiments were done four times with a representative experiment shown.

acid labelling. Thus, TNFα (0·1 ng ml^{-1}) resulted — after 60 min — in a slightly lower PC, PS and PE cellular levels; but the cellular amount of SM, PIP and PIP_2 was — similar to the [^{3}H]-palmitic acid labelling — significantly lower, than in the controls (Table 1). In the earliest part of treatments (5 min) the relative amount of labelled PI and PA was lower than in the controls, but after 60 min (PA) and 30 and 60 min (PI) the ^{32}P content was higher in these phospholipids than in the controls. In the case of SM, PIP and PIP_2 after 5-min treatments, the labelling was significantly

Table 1. The effect of TNFα on the phospholipid content of *Tetrahymena pyriformis* and the culture medium of the *Tetrahymena* populations. The cells were labelled with [^{32}P]-Na orthophosphate for 1 h. After labelling, the washed cells were inoculated into fresh culture medium or into 0·1 ng ml^{-1} TNFα-containing medium, and the cells were separated by centrifugation from the supernatant after 5, 15, 30 and 60 min. The lipids were separated from both the cells (a) and supernatant (b). The individual phospholipids were expressed as a percentage of the total incorporated ^{32}P which was taken as 100 per cent. The data represent averages (±SD) of three separate experiments.

	SM, PIP, PIP_2	PC, PS	PI	PA	PE
(a)					
Control 5 min	3·3 ± 0·11	28·2 ± 1·4	11·1 ± 0·85	5·0 ± 0·42	52·5 ± 3·8
Control 15 min	2·6 ± 0·09	25·5 ± 1·13	12·5 ± 1·1	7·0 ± 0·5	48·4 ± 4·0
Control 30 min	4·5 ± 0·13	26·5 ± 1·9	12·5 ± 0·9	5·7 ± 0·37	49·4 ± 4·8
Control 60 min	4·0 ± 0·11	30·3 ± 1·75	9·73 ± 0·7	3·37 ± 0·16	50·4 ± 3·8
TNF 5 min	11·6 ± 0·62*	29·6 ± 1·3	9·1 ± 1·1	1·5 ± 0·07*	46·8 ± 4·0
TNF 15 min	3·9 ± 0·14	26·27 ± 2·1	13·53 ± 0·85	7·35 ± 0·24	47·6 ± 3·88
TNF 30 min	3·2 ± 0·95	31·6 ± 1·8	16·45 ± 1·3†	4·15 ± 0·33	44·7 ± 5·2
TNF 60 min	2·4 ± 0·1†	27·7 ± 1·4	10·7 ± 0·97	7·0 ± 0·44*	47·6 ± 3·6
(b)					
Control 5 min	1·3 ± 0·06	28·2 ± 1·85	15·0 ± 0·67	—	55·5 ± 3·2
Control 15 min	1·0 ± 0·03	28·9 ± 1·23	12·7 ± 0·9	—	58·4 ± 4·11
Control 30 min	11·6 ± 0·74	23·1 ± 2·0	12·1 ± 1·0	—	53·2 ± 2·85
Control 60 min	5·0 ± 0·09	27·7 ± 1·11	9·7 ± 0·65	1·3 ± 0·023	56·3 ± 3·21
TNF 5 min	1·1 ± 0·06	28·4 ± 1·3	18·6 ± 1·15†	—	52·1 ± 3·05
TNF 15 min	—	23·4 ± 0·9	7·1 ± 0·77†	1·5 ± 0·02	68·1 ± 4·12*
TNF 30 min	4·9 ± 0·12*	30·1 ± 1·2†	8·1 ± 0·6†	1·9 ± 0·03	53·6 ± 2·95
TNF 60 min	5·8 ± 0·098	24·6 ± 1·06	8·8 ± 0·98	—	59·5 ± 3·18

* $p < 0·01$ compared to the control; † $p < 0·05$ compared to the control.

higher than in the controls. In the case of the culture medium some differences were also demonstrated — compared to the palmitic acid labelling.

Effect of TNFα on the Incorporation of ^{32}P into the Phospholipids of Tetrahymena

TNFα treatments resulted in significant alterations of the labelled PI content — compared to the controls: the 0·05 ng ml^{-1} TNFα after 15–30-min treatments significantly elevated the amount of PI, while after 60 min a significant decrease (Table 2) was observed. Treatments with 0·1 ng ml^{-1} TNFα decreased the PI level — except in the first 5 min of treatments. Apart from 1 and 60 min, treatments with 0·1 ng ml^{-1}, TNFα decreased or abolished the ^{32}P labelling of PIP_2 (Table 2).

Treatments with TNFα generally resulted in a reduced ^{32}P incorporation into the PC, PE and PA (Table 3), except for 60-min treatment with 0·05 ng ml^{-1} TNFα, where an elevation was found in the labelling of PE.

Effect of TNFα on the Growth Rate of Tetrahymena *Populations*

TNFα reduced growth intensity but the viability of cells did not differ from the controls as demonstrated by trypan blue exclusion (Figure 3). The lag phase of TNFα-treated populations was longer than that of the controls.

Effect of TNFα on the Chromatin Condensation of Tetrahymena

The short treatments (10 min) with 0·05 or 0·1 ng ml^{-1} TNFα did not cause any difference in the 546 nm light absorption (or transparency) compared to controls. Longer treatment (60 min) increased the light absorption at 546 nm (i.e. decreased the transparency), which indicates the condensation of chromatin (Figure 4).

Morphometric Analysis of the Tetrahymena *Cells*

The volume of the *Tetrahymena* cells was reduced significantly in the presence of both of the TNFα concentration tested after 60 min. The ratio of the shortest and longest axis (W/L) also decreased significantly, i.e. the cells were more slender than the control ones (Figure 5).

DISCUSSION

Many lipids or lipid-derived products generated in *Tetrahymena* by receptor-activated phospholipases

Table 2. Effect of TNFα on the ^{32}P incorporation into the inositol phospholipids of *Tetrahymena pyriformis*. The individual phospholipids were expressed as a percentage of the total incorporated ^{32}P which was taken as 100 per cent. The data represent averages (±SD) of three separate experiments.

	PI	PIP	PIP_2
Control 1 min	—	—	—
Control 5 min	0·7 ± 0·025	—	0·5 ± 0·0
Control 15 min	5·1 ± 0·3	1·7 ± 0·09	1·3 ± 0·023
Control 30 min	8·0 ± 0·41	3·0 ± 0·1	3·7 ± 0·1
Control 60 min	8·25 ± 0·71	4·4 ± 0·13	1·1 ± 0·05
TNFα 0·1 ng 1 min	1·7 ± 0·06	1·1 ± 0·05	1·3 ± 0·1
TNFα 0·1 ng 5 min	6·4 ± 0·17*	1·45 ± 0·04	—
TNFα 0·1 ng 15 min	—	1·3 ± 0·09	—
TNFα 0·1 ng 30 min	1·3 ± 0·09*	14·7 ± 1·1*	—
TNFα 0·1 ng 60 min	3·3 ± 0·12*	3·8 ± 0·078	—
TNFα 0·05 ng 1 min	5·1 ± 0·11†	—	7·0 ± 0·85†
TNFα 0·05 ng 5 min	1·7 ± 0·074†	3·1 ± 0·11‡	—
TNFα 0·05 ng 15 min	26·1 ± 1·25*	—	1·1 ± 0·0
TNFα 0·05 ng 30 min	21·25 ± 1·6*†	—	1·1 ± 0·11
TNFα 0·05 ng 60 min	3·05 ± 0·14*	1·6 ± 0·03*‡	3·4 ± 0·077*

* $p < 0·01$ compared to the control; † $p < 0·01$ compared to the 0·1 ng TNFα-treated cells; ‡ $p < 0·05$ compared to the 0·1 ng TNFα-treated cells.

Table 3. Effect of TNFα on the incorporation of ^{32}P into the phospholipids of *Tetrahymena pyriformis*. The individual phospholipids were expressed as a percentage of the total incorporated ^{32}P which was taken as 100 per cent. The data represent averages (±SD) of three separate experiments.

	PC	PE	PA
Control 1 min	—	—	—
Control 5 min	—	1·0 ± 0·008	—
Control 15 min	0·8 ± 0·0	4·9 ± 0·09	1·15 ± 0·078
Control 30 min	2·8 ± 0·09	14·9 ± 0·11	5·3 ± 0·3
Control 60 min	11·5 ± 1·02	21·45 ± 0·3	10·3 ± 0·87
TNFα 0·1 ng 1 min	—	0·5 ± 0·0	—
TNFα 0·1 ng 5 min	—	1·5 ± 0·02	—
TNFα 0·1 ng 15 min	1·4 ± 0·08†	5·75 ± 0·27	2·2 ± 0·083
TNFα 0·1 ng 30 min	1·5 ± 0·06†	13·3 ± 0·95	4·5 ± 0·09
TNFα 0·1 ng 60 min	7·75 ± 0·34*	23·7 ± 1·12	8·05 ± 0·13
TNFα 0·05 ng 1 min	—	2·0 ± 0·088	—
TNFα 0·05 ng 5 min	—	1·6 ± 0·05	—
TNFα 0·05 ng 15 min	0·8 ± 0·0‡	3·7 ± 0·12‡	1·7 ± 0·05
TNFα 0·05 ng 30 min	2·0 ± 0·087	13·2 ± 1·3	5·7 ± 0·16
TNFα 0·05 ng 60 min	7·4 ± 0·44*	25·7 ± 1·68†	7·45 ± 0·48†

* $p < 0·01$ compared to the control; † $p < 0·05$ compared to the control; ‡ $p < 0·05$ compared to the 0·1 ng TNFα-treated cells.

acting on membrane phospholipids are mediators and second messengers in the signalling processes.[17–19] The agonists and antagonists of these receptor-regulated enzymes play very important roles in the signalling and in the regulation of many metabolic pathways. Moreover they help in understanding the mechanism and importance of these systems in this unicellular organism. The main goal of our present work was to study — in *Tetrahymena* — the effect of TNFα on the phospholipid metabolism and on the generation of lipid second messengers, and to investigate mechanisms which could indicate favourable or disadvantageous effects of TNFα in this unicellular organism.

The experiments demonstrated that TNFα in *Tetrahymena* — similarly to certain mammalian cells — alters lipid metabolism. TNFα caused a slower breakdown of [^{3}H]-palmitic acid labelled PC, PI, PA and PE, as indicated by higher cellular content of these labelled phospholipids compared to the controls. In parallel with this phenomenon, the secretion of phospholipids was reduced, in the presence of TNFα.

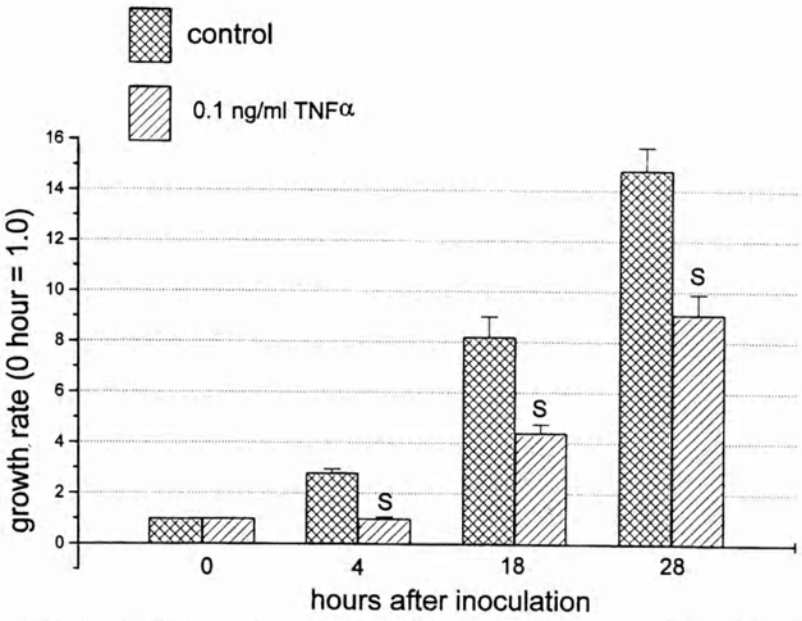

Figure 3. Effect of 0·1 ng ml^{-1} TNFα on the growth rate of *Tetrahymena pyriformis* populations. 5×10^4 cells were inoculated into fresh culture medium or TNFα-containing medium. The data represent the mean ± SD of four independent experiments. S = $p < 0.01$ compared to the control.

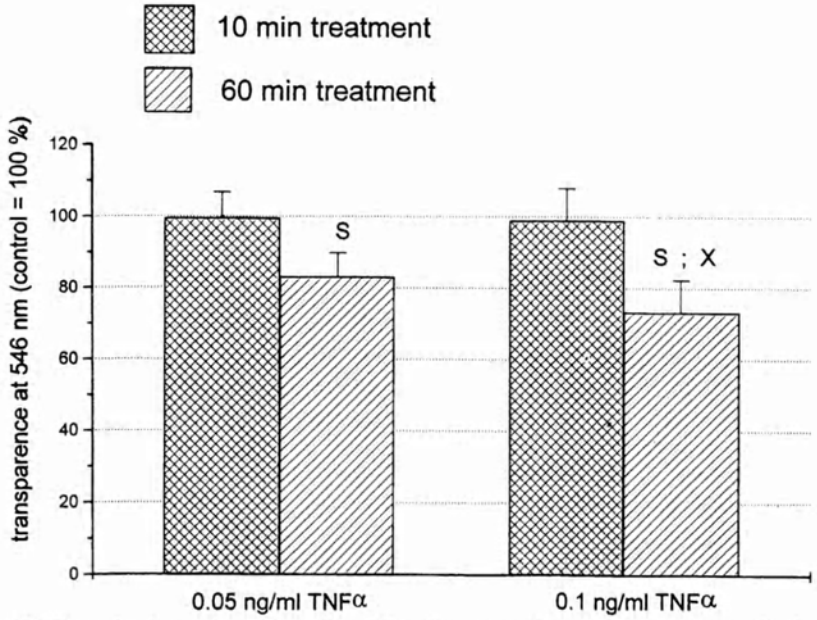

Figure 4. Effect of TNFα on the chromatin condensation of *Tetrahymena pyriformis*. The transparence (*9*) of nuclei were measured at 546 nm. The data represent the mean ± SD of four independent experiments. S = $p < 0.01$ compared to the control; X = $p < 0.05$ compared to the 0·05 ng ml^{-1} TNFα-treated cells.

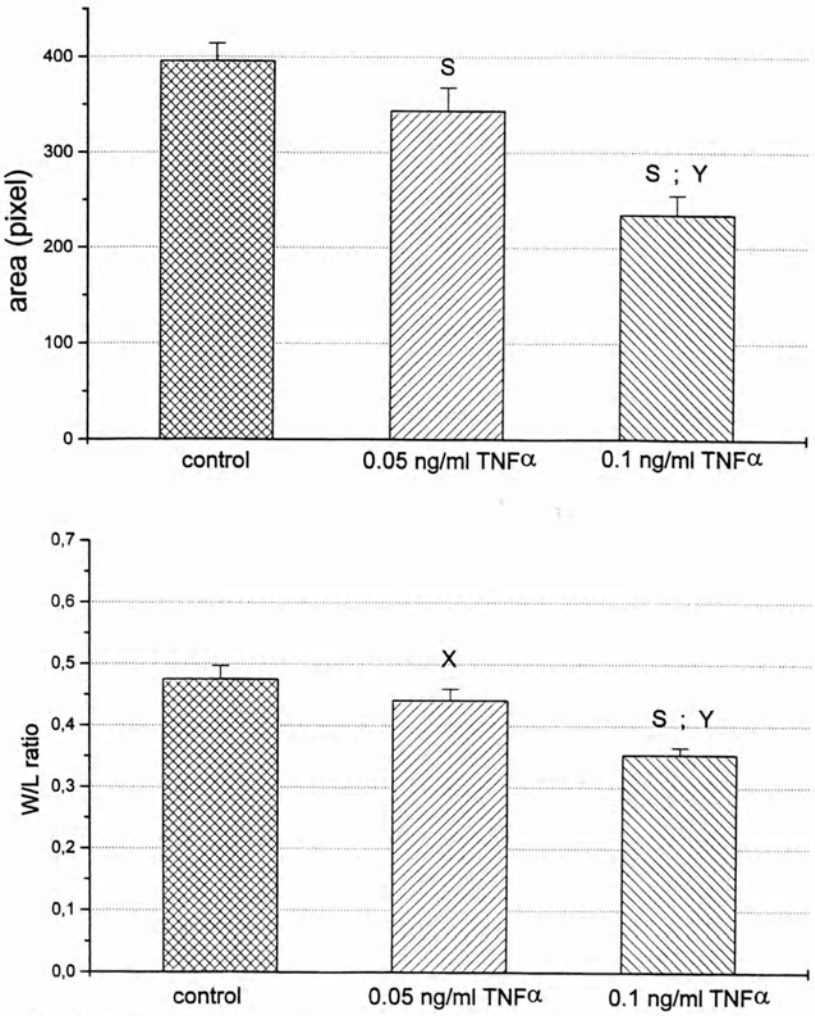

Figure 5. Effect of 60-min TNFα treatments on the morphological features of *Tetrahymena pyriformis*: (a) the area of the cells; (b) the W/L ratio. The data represent the mean of four independent measurements. One hundred cells were measured in each experimental group. S = $p < 0{\cdot}01$ compared to the control; X = $p < 0{\cdot}05$ compared to the control; Y = $p < 0{\cdot}01$ compared to the 0·05 ng ml^{-1} TNFα-treated cells.

TNFα is able to inhibit phospholipid synthesis/metabolism in many cell types, e.g. human pneumocytes[10] or Caco intestinal cells.[8] Similar situations are caused by TNFα in *Tetrahymena* in the case of PC, PI, PA and PE. It is known that TNFα-stimulated degradation of sphingomyelin results in an elevation of the ceramide content, and decrease of SM content. After [^{3}H]-palmitic acid labelling we measured reduced radioactivity in the chromatographic spots corresponding to the SM, and higher activity in ceramide containing spots.

The elevated ceramide content may be responsible for the reduced growth capacity of *Tetrahymena* populations and for the chromatin condensation and for the alteration of morphological features in the cells. These effects are expressed strongly after treatments with a cell-permeable ceramide analogue, *N*-acetyl-sphingosine (C_2-ceramide) (manuscript in preparation). For example the growth activity of *Tetrahymena* populations is completely inhibited by 10 μM C_2-ceramide treatments; the condensation of chromatin is stronger and the W/L ratio of the

cells indicates rounding of the cells. Our experience is that rounding of cells is a reaction to disadvantageous changes of life conditions.

The cells generally contain two PC and SM pools at the inner and outer leaflet of the plasma membrane. The so-called signalling pools are located at the inner leaflet of the membrane and TNFα caused PC and SM degradation of these pools[25] in experiments with mammalian cells. However at present we have no data on these pools in *Tetrahymena*. TNFα increases both secreted and cellular PLA_2 activity, and mediates the exocytosis of 14 kDa PLA_2.[26] In the culture medium of *Tetrahymena* PLA_1, triacylglycerol lipase[27] and acid PLC[28] as secreted. The PLA_2 content of the culture medium of *Tetrahymena* remains to be determined, although on the basis of the present experiments it is improbable that in the culture medium the secreted enzymes (e.g. PLA_2) are stimulated by TNFα and these enzymes could release ceramide from the outer leaflet of the plasma membrane. The improbability is supported by the fact that the amount of ceramide in the culture medium of TNFα-treated cells is lower than that of the controls.

The experiments with ^{32}P labelled phospholipids resulted in slightly different results compared with the [^{3}H]-palmitic acid labelled ones. In both cases the breakdown of PI and PA was decreased, and the secretion of these phospholipids was lower. However, in the level of ^{32}P labelled PE and PC, TNFα caused no significant alterations; and the secretion of these phospholipids were moderately decreased. The ^{32}P labelled PIP and PIP_2 content decreased similarly to the [^{3}H]-palmitic acid labelled cells, but the content of these inositol phospholipids in the culture medium was similar in both control and TNFα-treated cells.

The difference between [^{3}H]-palmitic acid and ^{32}P labelled phospholipid breakdown and secretion is probably a consequence of a different fatty acid content of these molecules. In the membrane PC and PE of *Tetrahymena pyriformis* WH-14 cells[29] the major fatty acid components are the $C18:1^{\Delta 9}$ (oleic acid), $C18:2^{\Delta 9,12}$ (linoleic acid), $C18:2^{\Delta 6,11}$ (cilienic acid) and $C18:3^{\Delta 6,9,12}$ (γ-linoleic acid), and they contain a relatively low level of C16:0 (palmitic acid). The fatty acid content and the nature of the fatty acids (i.e. degree of saturation, desaturation and the number of carbon atoms) in the phospholipids plays a very important role in numerous phenomena in response to changes in environmental conditions. Different treatments (e.g. ethanol) are able to modify the acyl group composition of membrane phospholipids. The most striking differences were detected in PE where there was a large increase in the level of C18:2 fatty acids.[29] The different breakdown and secretion of [^{3}H]-palmitic acid and ^{32}P labelled phospholipids may be a consequence of different fatty acid content: the ^{32}P labelled phospholipids contain the 'normal' fatty acids characteristic of *Tetrahymena*, while [^{3}H]-palmitic acid labelled fatty acids contain, of course, palmitic acid. We are able to measure the metabolism of [^{3}H]-palmitic acid-containing lipids, and these lipids probably have an altered metabolism compared to the C18-fatty acid containing phospholipids.

TNFα treatments generally resulted in a reduced ^{32}P incorporation into the phospholipids of *Tetrahymena*. TNFα in 0·1 ng ml^{-1} concentration totally inhibited the labelling of PIP_2, except for in the first 1 min of treatments; while in the case of PI in the first 5 min, and in PIP the first 5 and 30 min the ^{32}P content was elevated, compared with the controls. These facts indicate the inhibited metabolism of inositol phospholipids in presence of 0·1 ng ml^{-1} TNFα. This disturbed metabolism is characterized by the absence of labelling of PIP_2 in these groups. Similar results were obtained with PC, PA and PE: TNFα treatments reduced the incorporation of ^{32}P.

On the basis of the results it is obvious that TNFα inhibits the metabolism of phospholipids in *Tetrahymena*. The unfavourable effects (manifested in cell division, cell form and nuclear condensation) of TNFα would be caused by disturbed phospholipid metabolism, the inhibition of inositol phospholipid breakdown, the stimulation of ceramide formation and as a consequence of these, by impaired signalling. The effects of TNFα on *Tetrahymena* are similar to the effects of this cytokine on mammalian cells. Thus it is likely that the unicellular organism can receive the signal of TNFα and decipher it, in ways similar to those of the cells of higher organisms. This observation gives new data to our picture of the evolution of the signalling system.[30]

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