Cell proliferation status, cytokine action and protein tyrosine phosphorylation modulate leukotriene biosynthesis in a basophil leukaemia and a mastocytoma cell line

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Mast cells, mastocytoma cells and basophil leukaemia cells are well-established producers of leukotrienes when grown and stimulated appropriately. I report that the cells' ability to produce leukotrienes is dependent on the cells' proliferative status or their provision with growth factors. Proliferating MC/9 and subconfluent RBL2H3 cells respond maximally to stimulation by 1 μM ionomycin with the production of 56 and 32 pmol of cysteinyl-leukotrienes/10^6 cells respectively. In contrast, confluent RBL2H3 or growth-arrested MC/9 cells lose their ability to generate leukotrienes in response to ionomycin treatment. This rapid down-regulation of leukotriene synthesis is also observed when proliferating RBL2H3 cells are transferred to growth-factor-free medium, wherein cellular leukotriene-synthesis capacity has an apparent half-lifetime of 60 min. Transfer back into growth medium results in the regeneration of leukotriene synthesis capacity within 6 h. In growth-arrested MC/9 cells, leukotriene production ability can at least partially be restored by priming the cells with interleukin 3, but not with interleukin 4. In RBL2H3 cells, pretreatment with protein tyrosine kinase inhibitors such as genistein (5 min, 37 μM), herbimycin A (6 h, 3 μM) or tyrphostin 25 (16 h, 100 μM) completely inhibits leukotriene generation, whereas okadaic acid (15 min, 0.5 μM) has no effect. Under these conditions, both genistein and herbimycin A strongly impair ionomycin-induced protein tyrosine phosphorylation. Our study indicates that leukotriene generation in these tumour cells is tightly regulated by their proliferation status and supply with growth factors, and cell stimulation towards leukotriene synthesis appears to involve protein tyrosine kinase activity.

INTRODUCTION

The intracellular formation of leukotrienes (LTs) can be elicited in competent cells such as neutrophils, eosinophils and mast cells by a range of stimuli, including growth factors, antigens, chemotactic peptides and experimental agents such as Ca^{2+}-ionophores [1,2]. LTs themselves are recognized as effectors of high potency mediating increased vascular permeability, vasoconstriction, bronchoconstriction and leucocyte chemotaxis [1], and have therefore been suggested to contribute substantially to various inflammatory diseases [3,4]. Interestingly, LT actions have been demonstrated to include also the modulation of cellular proliferation [5].

Various steps in the intracellular enzymic sequence of events leading to LT synthesis are known in some detail. They include the Ca^{2+}-induced translocation of phospholipase(s) from the cytosol to a membrane site and release of arachidonate [6,7], and a similar translocation of the bifunctional enzyme 5-lipoxygenase/LTA_4 synthase [8,9] and its activation toward LTA_4 production, the latter of which depends on the presence of an activating protein [10].

The enzymic capacity for LT production is restricted to few cell types, and, depending on their specific enzymic pattern, they predominantly produce LTB_4 or cysteinyl-LTs, which include LTC_4 and its metabolites [2]. Modulation of a certain cell type's capacity for LT generation has been observed in various ways; for example, LT synthesis capacity is induced during dimethyl sulphoxide (DMSO)-elicited differentiation of HL60 cells [9,11–13]. It varies also depending on the efficacy of the *v*-myb oncogene product [14], and cells can be primed toward increased or diminished LT generation by cytokines, including various interleukins (ILs) [15–19]. In view of the biological potency of LTs, such alterations of the LT synthesis capacity may obviously affect the tissue or organism involved in the extracellular action of secreted LTs. In addition, LTs have also been suggested to act via intracellular signal transduction, leading to activated gene transcription [20–22] and increased cellular proliferation [5].

Since mastocytoma cells and basophilic leukaemia cells are established producers of LTs [2], I used such cells to investigate the question of whether their LT synthesis capacity is stably expressed irrespective of their proliferative status, or whether it is modulated due to alterations in growth-factor supply and other conditions affecting cell growth. I demonstrate that, in the cells studied, LT synthesis capacity strongly correlates with cell proliferation, and that this LT-generating system is rapidly down-regulated in cells growth-arrested by confluency or starvation, respectively. The loss of LT synthesis activity can be partially or fully restored by appropriate growth factors such as IL3 or by serum. In addition, as both the receptor-mediated action of IL3 [23,24] and the ionophore-induced Ca^{2+} stimulation of cells are known to result in intracellular protein tyrosine kinase (PTK) activation [25], I wondered whether PTK action was involved in regulating the enzymic pathway leading to LT production. In this study I provide evidence indicating that the intracellular signal pathway resulting in LT production depends on a functional PTK activity in the cells analysed.

MATERIALS AND METHODS

Cell culture

RBL2H3 cells were kindly provided by Dr. A. M. Gilfillan, Hoffmann–LaRoche (Nutley, NJ, U.S.A.), and originally came

Abbreviations used: DMSO, dimethyl sulphoxide; IL, interleukin; LT, leukotriene; PTK, protein tyrosine kinase; r.i.a., radioimmunoassay.

from Dr. M. Beaven, NIH (Bethesda, MD, U.S.A.). The mouse mast-cell clone MC/9 was obtained from American Type Culture Collection (Rockville, MD, U.S.A.). RBL2H3 cells were maintained attached to 175 cm^2 tissue-culture flasks (Falcon; Becton Dickinson, Lincoln Park, NJ, U.S.A.) in Eagle's Minimum Essential Medium (Gibco, Grand Island, NY, U.S.A.) supplemented with 10% (v/v) heat-inactivated fetal-calf serum (Gibco), penicillin (100 units/ml) and streptomycin (100 mg/ml; Gibco) in a humidified atmosphere of air/CO_2 (19:1) at 37 °C. For subcultivation, cells were dislodged from the flask with trypsin (0.05%)/EDTA (0.02%) solution (Gibco) and re-plated after rinsing. For experiments, the cells were re-plated as indicated at a density of 5×10^5 or 1×10^6 cells in 1.5 ml of medium in 35 mm-diameter tissue-culture dishes (Falcon) for 16–18 h; at that time point, these RBL2H3 cultures had reached approx. 50% or 100% confluency, respectively, and were used for stimulation experiments.

MC/9 cells were maintained in Dulbecco's modified Eagle's medium (Gibco) containing 10% heat-inactivated fetal calf serum, penicillin (100 units/ml), streptomycin (100 mg/ml), 50 μM β-mercaptoethanol (Gibco), and 5% (v/v) of conditioned medium from transformed X63Ag8-653 myeloma cells, which constitutively secrete large quantities of recombinant murine ILs (IL2–5) [26]. MC/9 cells were kept in culture flasks at a density between 5×10^4 and 3×10^5 cells/ml by appropriate dilution. For starvation, MC/9 cells were seeded at 1×10^5 cells/ml of medium, which differed from normal growth medium by containing only 2% fetal-calf serum and no conditioned medium. Cellular viability was monitored by using Trypan Blue and was always > 95%.

Stimulation of LT synthesis

Adherent RBL2H3 cells at appropriate densities were rinsed twice with prewarmed (37 °C) incubation buffer, containing 137 mM NaCl, 2.7 mM KCl, 0.4 mM Na_2HPO_4, 5.6 mM glucose, 10 mM Hepes, 1.8 mM $CaCl_2$ and 1.3 mM $MgSO_4$, pH 7.4. Cells were stimulated at 37 °C with gentle agitation in 1 ml of incubation buffer by addition of ionomycin (1 μM; Sigma, St. Louis, MO, U.S.A.) or its vehicle, DMSO. Stimulation was stopped after the indicated time periods by putting cells on ice and removing the cell supernatant buffer into 4 ml of ice-cold methanol containing 1 mM 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (Sigma); this mixture was analysed for secreted LTs. The adherent cell layer was rinsed with 2×1 ml of ice-cold buffer (20 mM Tris/HCl, pH 7.5, 0.5 M NaCl), then extracted for intracellular LTs by lysing cells with 100 μl water for 5 min at 0 °C, by addition of 800 μl of methanol/1 mM 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl, and by centrifugation of the methanolic mixture at 10000 ***g*** for 10 min at 4 °C; the supernatant was analysed for intracellular LTs.

Incubation and analogous stimulation of MC/9 cells (1×10^7 cells/ml) by ionomycin was performed at 37 °C in a total volume of 700 μl of incubation buffer. After the indicated time periods, cells were centrifuged for 10 min at 150 ***g*** and 4 °C, the cell-free supernatant mixed with 4 vol. of ice-cold methanol/1 mM 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl and analysed for secreted LTs. The cell pellet was lysed and extracted for intracellular LTs as described above.

Secreted or intracellular cysteinyl-LTs (LTC_4, LTD_4, LTE_4) in methanolic supernatant or cell extracts, respectively, were individually quantified by radioimmunoassay (r.i.a.) after reversed-phase h.p.l.c. separation as previously described [27] or by direct r.i.a. of dried extract samples without prior h.p.l.c. as indicated in the text. Correction was made for the recoveries and cross-reactivities of individual LTs in the r.i.a. (relative percentage cross-reactivities: LTE_4, 100; LTD_4, 160; LTC_4, 219) as reported previously [28].

SDS/PAGE and immunoblot analysis of tyrosine-phosphorylated proteins

After pretreatment and stimulation of cells, the culture dish was placed on ice and the supernatant was aspirated. The adherent cultures were washed twice with 1 ml of ice-cold buffer (20 mM Tris/HCl, pH 7.5, 0.5 M NaCl), and then lysed in 0.1 ml of lysis buffer containing 50 mM Hepes, pH 7.4, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 100 mM NaF, 10 mM sodium pyrophosphate, 0.1 mM phenylmethanesulphonyl fluoride, 40 μg/ml each of leupeptin and aprotinin (Sigma), 0.8 mM sodium orthovanadate, 30 mM *p*-nitrophenyl phosphate (Sigma) and 1% Triton X-100. The cell lysates were scraped from the dish and centrifuged at 12000 ***g*** for 10 min at 4 °C. The supernatant was mixed with an equal volume of a buffer containing 0.06 M Tris, pH 6.8, 10% glycerol, 2% SDS, 0.01% Bromophenol Blue and 0.05% β-mercaptoethanol, and the mixture was boiled for 5 min. The samples (10–50 μg of protein) were separated by SDS/PAGE on a 8%-acrylamide gel and electrophoretically transferred to nitrocellulose paper. Immunoblotting was performed with the ^{125}I-labelled anti-phosphotyrosine monoclonal antibody 4G10 (UBI, Lake Placid, NY, U.S.A.), which was radiolabelled by the Iodogen method (Pierce, Rockford, IL, U.S.A.) according to the manufacturer's recommendations. Tyrosine-phosphorylated proteins were revealed by autoradiography at −70 °C using XAR-5 film. Protein was determined by Peterson's modification of the micro-Lowry method (Sigma) with BSA as standard.

Statistical analysis

Student's two-tailed *t* test was used to analyse differences for significance. Data are given as means ± S.D. or S.E.M.

RESULTS

Biosynthesis of LTs in RBL2H3 cells and MC/9 cells

Stimulation of sub-confluent proliferating RBL2H3 cells with ionomycin resulted in a time- and dose-dependent release of cysteinyl-LTs: increased LT formation was detectable after at least 5 min, reached its maximal amount within 20 min, and stayed at this level for up to 1 h (results not shown). Stimulation for 20 min with ionomycin at a concentration of 0.05, 0.1, 0.5 and 1.0 μM resulted in the average production and secretion of 1, 3, 19 and 32 pmol of cysteinyl-LTs/10^6 cells, equivalent to about 80 fmol of endogenous LTs/μg of protein (Table 1, left column). When the RBL2H3 cells were allowed to grow to confluency, they no longer were able to respond to ionomycin stimulation with LT production (Table 1, right column). There was no apparent difference in viability between non-confluent and confluent cells, as judged by Trypan Blue exclusion.

In proliferating MC/9 cells, maximal LT production was observed within 20 min after stimulation by 1 μM ionomycin, and this production amounted to 56 pmol of cysteinyl-LTs/10^6 MC/9 cells. In both cell lines, almost all of the generated cysteinyl-LTs were secreted into the extracellular medium; under no condition tested did we find more than 5% of the total LTs remaining in the cells. Tracer studies using exogenous [$^3H_8$]LTC_4 and subsequent h.p.l.c. analyses of radiolabelled and of endogenously formed LTs indicated that MC/9 cells only produce LTC_4, but do not metabolize it further during our experimental time periods. In contrast, most of the exogenous or endogenous

Table 1 LT production in sub-confluent and confluent RBL2H3 cells

Adherent cells at indicated confluency were rinsed twice and stimulated in incubation buffer with 1 μM ionomycin or as control with DMSO vehicle for 20 min at 37 °C. Endogenous LTE_4 production was determined by r.i.a. analyses of cell supernatants. Data are means ± S.E.M. from three separate experiments done in triplicate dishes.

	LT production (pmol of $LTE_4/10^6$ cells)	
Stimulation	At 50% confluency	At 100% confluency
Control (DMSO)	0.5 ± 0.5	0.7 ± 0.5
Ionomycin	32.0 ± 1.0	1.7 ± 0.0

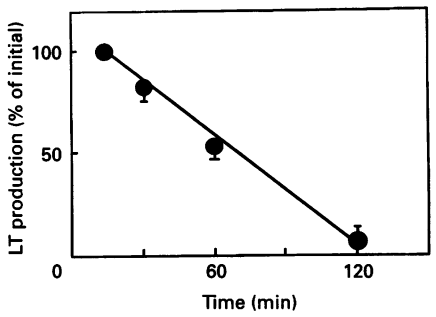

Figure 1 Time-dependent loss of LT synthesis capacity in growth-medium-deprived RBL2H3 cells

Adherent cells at approx. 50% confluency were rinsed twice and kept for the indicated time periods in incubation buffer before stimulation with 1 μM ionomycin for 20 min at 37 °C. Endogenous LT production was determined as LTE_4 by r.i.a. analyses of cell supernatants. Data are means ± S.E.M. from three separate experiments done in triplicate dishes.

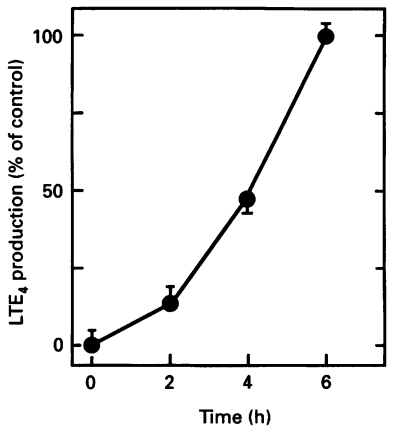

Figure 2 Growth-medium-induced regeneration of LT synthesis capacity in RBL2H3 cells

Adherent cells at approx. 50% confluency were rinsed twice and kept in incubation buffer for 2 h. After detachment, washing, and re-plating of cells in growth medium for the indicated time periods, stimulation of LT production was performed with 1 μM ionomycin for 20 min at 37 °C. Data are means ± S.D. from two separate experiments done in triplicate dishes.

LTC_4 in RBL2H3 cells is catabolized to LTE_4 under our conditions (results not shown). Thus, endogenous production of cysteinyl-LTs in MC/9 cells could be measured as LTC_4 by direct r.i.a., whereas in RBL2H3 cells direct r.i.a. measurements of LTE_4 are given after correction for the minor fractions of LTC_4 and LTD_4 present in these samples.

Table 2 Priming influence of IL3 and IL4 on LT synthesis capacity in growth-arrested MC/9 cells

Cells were starved for 16 h (see the Materials and methods section) before resuspension at a density of 10^7 cells/ml in Dulbecco's modified Eagle's medium containing 20 mM Hepes, pH 7.4. Priming with vehicle (PBS), IL3 or IL4 (50 ng/ml each) for 30 min was followed by stimulation with 1 μM ionomycin or vehicle (DMSO) for 20 min at 37 °C. LTC_4 production was determined by r.i.a.; values indicate means ± S.D. from two separate experiments done in triplicate.

Priming	Stimulation	LT synthesis (pmol/10^6 cells)
PBS	Ionomycin	2.0 ± 0.2
IL3	Ionomycin	8.0 ± 0.4
IL4	Ionomycin	3.1 ± 0.5
IL3	DMSO	1.8 ± 0.3
IL4	DMSO	2.1 ± 0.5

Stability and regeneration of LT synthesis capacity in RBL2H3 cells

Proliferating subconfluent RBL2H3 cells generated large amounts of LTs (Figures 1 and 2). However, they lost this capacity quickly if the growth medium was replaced by incubation buffer (Figure 1); the net half-lifetime of the enzymic system required for arachidonate release and subsequent LT synthesis appeared to be approx. 60 min (Figure 1). After 2 h in incubation buffer, the LT synthesis ability of the cells had disappeared almost completely (Figure 1), although the cells showed no sign of decreased viability at that time point.

When such growth-medium-deprived RBL2H3 cells were put back into growth medium and stimulated towards LT generation, their LT synthesis capacity gradually recovered with time, was back to normal after 6 h (Figure 2), and stayed the same at 8 h and 10 h (results not shown).

Cytokine priming of LT synthesis in MC/9 cells

Since the exact growth-factor requirements necessary for RBL2H3 cell proliferation have not been defined, I used the IL3-dependent MC/9 cell line to see whether cytokines necessary for cellular proliferation are also involved in the modulation of the LT synthesis apparatus. When proliferating MC/9 cells were growth-arrested by starvation, they almost completely lost their capacity for LT production, as compared with normal LT generation in MC/9 cells (Table 2). Priming of such starved cells with their growth factor IL3 (recombinant murine IL3, 50 ng/ml; Bachem, Bubendorf, Switzerland) for 30 min partially restored their ability to release LTs after ionomycin stimulation. In contrast, the same concentration of IL4 (recombinant murine IL4, 50 ng/ml; Genzyme, Cambridge, MA, U.S.A.) only weakly primed these cells towards LT generation ($P < 0.05$, compared with PBS-treated controls), but less efficiently than IL3. In the absence of ionomycin neither IL elicited LT formation (Table 2).

Involvement of PTK activity in LT synthesis

Since IL3 primes and Ca^{2+}-ionophore elicits LT generation, and are known to include in their effects the activation of intracellular PTK [23–25], I investigated the possibility that the

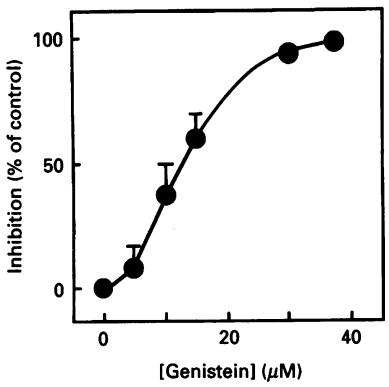

Figure 3 Dose-dependent inhibition by genistein of LT generation in RBL2H3 cells

Adherent cells at approx. 50% confluency were rinsed twice and incubated in incubation buffer for 5 min with genistein at the indicated concentrations before stimulation with 1 μM ionomycin for 20 min at 37 °C. LT synthesis was determined by measuring LTE_4 in cell supernatants by r.i.a. Data are means ± S.D. from two experiments done in triplicate dishes.

Table 3 Action of PTK or protein phosphatase inhibitors on LT generation in RBL2H3 cells

Adherent cells at approx. 50% confluency were treated with the compounds or their vehicle (control) for the indicated time period in incubation buffer (*) or in growth medium (†) before stimulation in incubation buffer with 1 μM ionomycin for 20 min at 37 °C. LT synthesis was determined by measuring LTE_4 by r.i.a. Data are given as means ± S.D. from at least two separate experiments per condition done in triplicate dishes.

Pretreatment			LT synthesis (% of control)
Genistein:	5 min,	30 μM*	5±2
Herbimycin A:	5 min,	3 μM*	99±3
	6 h,	3 μM†	2±2
Tyrphostin 25:	16 h,	100 μM†	1±1
Okadaic acid:	15 min,	0.5 μM*	97±3

synthesis of cysteinyl-LTs depends on protein tyrosine phosphorylation. Using the effective PTK inhibitor genistein (4′,5,7-trihydroxyisoflavone; Sigma) [29], I observed a dose-dependent inhibition of LT generation (Figure 3). Preincubation of RBL2H3 cells with 37 μM genistein for 5 min resulted in a complete blockade of the ionomycin-inducible LT production (Figure 3); the half-maximal inhibitory effect (IC_{50}) of genistein was at about 12 μM (Figure 3). Other PTK-inhibitory compounds such as herbimycin A (Gibco) [30,31] and tyrphostin 25 (Gibco) [32] also effectively prevented such LT formation (Table 3), albeit either after longer preincubation (herbimycin A, 6 h) or at higher dosage (tyrphostin 25); in view of the short half-lifetime of the LT synthesis capacity with RBL2H3 cells in incubation buffer (Figure 1), these prolonged pretreatments of cells with herbimycin A and tyrphostin 25 were performed with the cells in growth medium. The observed effects of genistein, herbimycin A and tyrphostin 25 were all due to inhibition of LT synthesis rather than to impairment of LT secretion into the extracellular medium, since I did not observe any increased intracellular accumulation of LTs under these inhibitory conditions. Pretreatment of cells for 5 min with either herbimycin A (Table 3) or tyrphostin 25 did not impair LT synthesis in RBL2H3 cells. The apparent differences in the time needed for an inhibitory action of the compounds used may be due to their different ability to reach their intracellular target or, in the case of herbimycin A, to act efficiently on PTK degradation. On the other hand, the inhibition of phosphoprotein phosphatase(s) by okadaic acid (Biomol, Plymouth Meeting, PA, U.S.A.) [33,34] did not influence the apparent ability of ionomycin to elicit cellular LT production (Table 3). None of these compounds caused a loss in cellular viability during the experimental time periods.

Genistein and herbimycin A at doses effective in preventing LT formation also caused a significant decrease in ionomycin-induced protein tyrosine phosphorylation. Preliminary immunoblot data indicate that under our conditions ionomycin treatment resulted in the increased phosphorylation predominantly of a protein of molecular mass approx. 120 kDa; other proteins, of approx. 110 kDa, 90 kDa, 70 kDa, and 60 kDa, were also tyrosine phosphorylated (results not shown). The inhibitory actions of herbimycin A after 6 h pretreatment at 3 μM and of genistein after 5 min at 30 μM similarly affected apparently all PTK substrates visible in the immunoblots under each condition.

DISCUSSION

I have used the established LT-producing cell lines RBL2H3 [35] and MC/9 [36,37] as model systems to investigate the following: (i) the activity of the LT-synthesizing apparatus during different cellular growth phases, (ii) its sensitivity to growth factors, and (iii) the intracellular signal-transduction pathway effective during net generation of cysteinyl-LTs.

My studies show that, as long as these cells are proliferating and kept under conditions providing sufficient growth factors, they are able to generate LTs upon ionophore stimulation. However, when adherently growing RBL2H3 cells essentially stop proliferating on reaching confluency, the LT synthesis capacity is lost even in the continuing presence of growth-factor-containing medium. Growth arrest of MC/9 cells by serum deprivation also results in an almost complete down-regulation of their LT-generating system. Thus, although growth arrest is brought about in quite a different way for each cell line studied, our results indicate that one or several crucial components necessary in the sequence between arachidonate release and LTC_4 synthesis are strictly correlated with a proliferating status of these cells. Clearly, this novel down-regulation of LT synthesis capacity is not due simply to a decreased viability of the cells, nor is it due to a lack of energy sources; preliminary data also exclude a lack of GSH as a probable cause for the loss of LT synthesis capacity. Interestingly, stopping proliferation, as induced during HL60-cell differentiation, has been reported to have quite the opposite effect, namely an up-regulation of LT synthesis capacity [11–13] accompanied by increased appearance *de novo* of the mRNA for 5-lipoxygenase [38]. Thus, depending on the particular cell system, LT synthesis capacity either can parallel or is inversely correlated with cellular proliferation. It will be interesting to see whether in RBL2H3 and/or MC/9 cells LT synthesis and action are causally related to the proliferation of these cells. Investigations using specific inhibitors of LT synthesis and LT receptor antagonists are currently under way to test this possibility.

I have so far no evidence as to which intracellular factor or enzyme may be responsible for the apparent fast down-regulation of LT synthesis in the cells studied. The overall 1 h half-lifetime of the LT synthesis system in RBL2H3 cells is, however, much shorter than the reported 26 h half-lifetime of 5-lipoxygenase in HL60 cells [9]. Other components necessary for the net LT release, i.e. enzymes such as phospholipase A_2 [39] and LTC_4

synthase [40] or activating factors such as phospholipase-A_2-activating protein [41] and 5-lipoxygenase-activating protein [42], are conceivable direct or indirect targets of this short-term regulation.

In contrast with growth arrest, addition of appropriate growth factors or of growth-factor-containing medium quickly induces LT synthesis capacity in RBL2H3 and MC/9 cells respectively. Whereas IL3 has been shown to enhance further the LT synthesis activity in basophils that are already producing LT [15], we present evidence that IL3 can by itself switch a cellular system from an essentially non-LT-producing condition to a state with at least partial LT synthesis capacity. This action of IL3 cannot be accomplished equally well by IL4, and IL4 is not synergistic with IL3 in this respect (results not shown), as has been shown in the case of mast-cell proliferation [43]. Since ionomycin was used to stimulate the LT synthesis capacity in our studies, the inductive action of IL3 most likely modulates an intracellular factor rather than the IL3 receptor itself. It will be interesting to see whether prolonged incubation with IL3 alone is sufficient for complete restoration of LT synthesis capacity in MC/9 cells, as is the case within 6 h for regular growth medium (results not shown).

Pharmacological evidence from our studies suggests that phosphorylation of protein tyrosine residues by intracellular PTK activity represents an essential part in the ionophore-induced chain of signal transduction leading to cysteinyl-LT generation. Three lines of evidence support this notion: (i) PTK activity is increased during ionomycin action, (ii) PTK inhibitors at doses preventing cysteinyl-LT synthesis also effectively block ionophore-induced protein tyrosine phosphorylation, and (iii) the inhibitory action of PTK inhibitors such as genistein on cysteinyl-LT synthesis is dose-dependent. However, a contribution of the PTK inhibitors' antioxidant activity to the observed prevention of LT formation cannot be ruled out at present. So far, the compounds used have been demonstrated to affect tyrosine phosphorylation of various proteins, including membrane-associated receptors as well as cytosolic proteins [29–31]. As to the possible targets of PTK activity during LT generation in RBL2H3 cells, several phosphorylated proteins are candidates which partially correspond to substrates of PTK activity found in A23187- or antigen-stimulated RBL2H3 cells [25]. Any of these tyrosine-phosphorylated proteins potentially takes part in different enzymic steps modulating cysteinyl-LT generation. It remains to be demonstrated whether one of them is a cytosolic phospholipase A_2 under the control of PTK activity, as was shown for phospholipase Cγ [44]. So far, direct phosphorylation after growth-factor stimulation of a cytosolic phospholipase A_2 has been shown to be restricted to serine residues [45].

In summary, the present study illustrates a novel down-regulation of LT synthesis upon growth arrest in RBL2H3 and MC/9 cells. Importantly, restoration of growth conditions or supply with IL3 causes a rapid recovery of the LT synthesis system. I provide new evidence that the intracellular signal pathway responsible for cysteinyl-LT formation may be mediated through PTK-dependent phosphorylation. The identification and functional characterization of these phosphoproteins should provide important clues to the regulation of LT generation in competent cells.

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