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Stimulatory Effect of Interleukin-1α on Proliferation through a Ca^{2+}/Calmodulin-dependent Pathway of a Human Thyroid Carcinoma Cell Line, NIM 1

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NIM 1 cells, a human thyroid cell line established from a patient with thyroid papillary adenocarcinoma, produce cytokines such as interleukin-1α (IL-1α) and granulocyte-colony stimulating factor. In the present study, we investigated the signal transduction pathway in the proliferation of NIM 1 cells evoked by IL-1α. Incubation of NIM 1 cells with IL-1α for 48 h increased the incorporation of ^{3}H-thymidine (^{3}H-TdR). The stimulatory effect of IL-1α was evident at 0.01 ng/ml and the maximal effect was seen at 10 ng/ml. IL-1α evoked an influx of ^{45}Ca into NIM 1 cells within 3 min in a concentration-dependent manner (0.01–1 ng/ml). These stimulatory effects of IL-1α on both ^{3}H-TdR incorporation and ^{45}Ca influx were similarly inhibited by nicardipine, an inhibitor of voltage-dependent Ca^{2+} channels, in a concentration-dependent manner (10–1000 n*M*). The stimulatory effect of IL-1α on ^{3}H-TdR incorporation was inhibited by N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), an antagonist of calmodulin, but not by 1-(5-isoquinoline sulfonyl)-2-methylpiperazine (H-7), an inhibitor of protein kinase C. While the culture medium initially contained 0.75 m*M* Ca^{2+}, inhibition of ^{3}H-TdR incorporation by nicardipine and W-7 under these baseline conditions was also recognized. These results suggest that IL-1α stimulates cell proliferation through a Ca^{2+}/calmodulin-dependent pathway in NIM 1 cells.

Key words: Thyroid carcinoma — Interleukin-1α — Calcium — Calmodulin — Cell proliferation

Interleukin 1 (IL-1) is a proinflammatory cytokine which is produced by activated mononuclear phagocytes and other cells. There are two homologous forms of IL-1, α and β, which mediate many features of inflammation, such as fever, the acute phase response, leukocyte accumulation, and tissue destruction, by acting on a variety of cell types.[1,2] IL-1 has the capacity to affect cell growth and differentiation in various cell types. It has a stimulatory activity on the growth of fibroblasts,[3] mesangial cells,[4] osteoblast-like cells and chondrocytes,[5] astrocytoma cell line,[6] B cell line,[7] adult T-cell leukemia (ATL) cells[8] and gastric carcinoma cells.[9] On the other hand, IL-1 inhibits the growth of a human papillary thyroid carcinoma cell line[10] and a breast carcinoma cell line MCF-7.[11]

The NIM 1 cell line is a human thyroid cell line established from a patient with thyroid papillary adenocarcinoma associated with hypercalcemia and peripheral neutrocytosis, and produces multiple cytokines including IL-1α, IL-1β, IL-6, IL-11, granulocyte-colony stimulating factor (G-CSF), and granulocyte/macrophage-CSF (GM-CSF).[12] Previously, we reported the following characteristic properties of NIM 1 cells[13]: (A) mRNA expression of IL-1α and IL-1β and their production in the cell lysate and culture supernatant, (B) specific binding site for IL-1α, (C) neither cell proliferation nor cyclic AMP production in response to TSH and (D) stimulatory effects of IL-1α and IL-1β on cell proliferation, which were inhibited by the addition of anti-IL-1α and IL-1β antibodies. Based on these results, we proposed that IL-1 has a regulatory role in the growth of NIM 1 cells by an autocrine mechanism.[13] We have now further examined the intracellular mechanism of IL-1α-induced cell proliferation in NIM 1 cells.

MATERIALS AND METHODS

Materials Fetal calf serum (FCS) and RPMI 1640 medium were purchased from GIBCO Oriental Co., Ltd. (Tokyo). Cell culture plates were purchased from Becton Dickinson Company (Falcon, Lincoln Park, NJ), Corning (Corning, NY), and Costar (Cambridge, MA). Recombinant human IL-1α was kindly donated by Dr. M. Yamada (Dainippon Pharmaceutical Co., Osaka). IL-1α was produced by using *Escherichia coli* transduced with IL-1α cDNA[14] and was purified by gel filtration, ion-exchange chromatography, and high-pressure liquid chromatography. Nicardipine was provided by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo). H-7 (1-(5-isoquino-

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line sulfonyl)-2-methylpiperazine) and W-7 (N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide) were purchased from Seikagaku Kogyo Co., Ltd. (Tokyo). $CaCl_2$ and forskolin were purchased from Wako Pure Chemical Industries Ltd. (Osaka). [Methyl-^{3}H]thymidine (^{3}H-TdR) (5 Ci/nmol) and $^{45}CaCl_2$ (0.5–2 Ci/nmol) were purchased from Amersham Japan (Tokyo). [γ-^{32}P]ATP (3000 Ci/nmol) was purchased from New England Nuclear (Boston, MA).

NIM 1 cells NIM 1 cells used in this experiment were kindly donated by Dr. K. Endo (Kyoto University Medical School, Kyoto). This cell line was established from a metastatic deposit in a patient with papillary adenocarcinoma of the thyroid associated with hypercalcemia and peripheral neutrocytosis by the method of limiting dilution.[15] The morphological appearance of a specimen prepared from this metastatic deposit of the patient consisted of a well differentiated adenocarcinoma intermingled with poorly differentiated adenocarcinoma, and the synthesis of human thyroglobulin was confirmed immunohistochemically.[13]

Cell cultures NIM 1 cells were maintained by culturing in a RPMI 1640 medium containing $CaCl_2$ (0.75 m*M*), 10% FCS, penicillin (5×10^4 U/liter; Flow Laboratories, North Ryde, Australia), and streptomycin (50 mg/liter; Flow Laboratories) in plastic culture flasks at 37°C in 5% CO_2 and 95% air.[13] After the culture, the cells were detached by treatment with trypsin (0.25% trypsin; Sigma, St. Louis, MO), washed with phosphate-buffered saline (PBS) and seeded.

Growth assay of NIM 1 cells NIM 1 cells (1×10^4/well) were plated in 96-well culture plates (Falcon 3072; Becton Dickinson) and grown in RPMI 1640 medium without FCS, but with various concentrations of test agents (IL-1α, nicardipine, H-7 and W-7) at 37°C for 2 days in 5% CO_2 and 95% air.[13] After 2 days, when the cells were in the exponential growth phase, the cells were pulsed for another 24 h with 0.5 μCi of ^{3}H-TdR. Then the medium was aspirated, and the cells were detached from the plates with a solution of trypsin (0.25%) in PBS. The cells were harvested on glass fiber filters with an automatic cell harvester (Abekagaku Co., Ltd., Chiba). The radioactivity of each sample was determined by a liquid scintillation counter (LSC-3500E, ALOKA Co., Ltd., Tokyo). The cell proliferation is expressed as cpm of ^{3}H-TdR incorporated into the cells.

Influx of ^{45}Ca into NIM 1 cells NIM 1 cells were cultured (2×10^5 cells/dish) (Falcon 3001; Becton Dickinson) and grown in RPMI 1640 medium containing 1% FCS. The culture medium was removed by aspiration and the cells were washed twice with 1 ml of ice-cold Krebs-Ringer phosphate buffer. Krebs-Ringer phosphate buffer was composed of (n*M*): NaCl 154, KCl 5.6, $MgSO_4$ 1.1, $CaCl_2$ 2.2, NaH_2PO_4 0.85, Na_2HPO_4 2.15 and glucose 10, adjusted to pH 7.4. Then the cells were incubated at 37°C for 3 min in an environmental incubator-shaker (G24, New Brunswick Scientific Co., Inc., Edison, NJ) with 1.5 μCi of $^{45}CaCl_2$ in 1.0 ml of the medium in the absence or presence of IL-1α and nicardipine. After incubation, the cells were rapidly washed 4 times with 1 ml of ice-cold Krebs-Ringer phosphate buffer, then detached and solubilized in 1 ml of 10% Triton X-100, and the radioactivity was counted in a toluene-based scintillator using a liquid scintillation counter (LSC-3500E, ALOKA Co., Ltd.).[16] The influx of $^{45}Ca^{2+}$ was expressed as nmol/2×10^5 cells, being calculated from the initial specific radioactivity in the incubation medium.

Measurement of cyclic AMP NIM 1 cells were cultured (1×10^6 cells/dish) (Falcon 3001; Becton Dickinson) and grown in RPMI 1640 medium containing 10% FCS. After 2 days the medium was removed, and replaced with 1 ml of RPMI 1640 medium plus 1 m*M* 3-isobutyl-1-methylxanthine (IBMX) (Sigma). The medium was preincubated at 37°C for 10 min in 5% CO_2, and then incubated for another 10 min with or without a test compound (IL-1α or forskolin) in the presence of IBMX (1 m*M*). After aspiration of the medium, the cells were rapidly scraped into ice-cold 7% trichloroacetic acid (TCA) and centrifuged. The supernatant fraction was extracted with 2 ml of water-saturated diethyl ether 2 times. After aspiration of the diethyl ether solution, the aqueous extract was applied to an ion exchange column (AG 50W×4, H^+ type).[16] Cyclic AMP in the effluent was assayed using a cyclic AMP assay kit (Amersham Japan).

Assay of protein kinase C activity Protein kinase C was assayed by measuring the incorporation of ^{32}P into histone (type III-s, Sigma) from [γ-^{32}P]ATP.[17] After incubation for 5 min at 37°C with or without IL-1α (1 ng/ml), cells (2×10^6) were scraped into 2 ml of ice-cold buffer (5 m*M* EGTA; 2 m*M* dithiothreitol; 1 m*M* phenylmethylsulfonyl fluoride; 15 m*M* HEPES, pH 7.4), homogenized, and centrifuged at 105,000*g* for 20 min at 4°C. The resultant supernatant was used as cytosol enzyme. The pellet was resuspended in 200 μl of homogenizing buffer containing 1% Triton X-100, incubated at 4°C for 20 min, and centrifuged at 105,000*g* for 20 min; the resultant supernatant was used as a particulate enzyme. Protein kinase C activity was measured in a reaction mixture (0.2 ml) containing 10 μM [γ-^{32}P]ATP (5–10$\times$ 10^5 cpm), 50 μg/ml histone, enzyme (1–1.5 μg protein), 5 m*M* $MgCl_2$, 0.5 m*M* EGTA, 2 m*M* dithiothreitol, 1 m*M* phenylmethylsulfonyl fluoride, 15 m*M* HEPES (pH 7.4) with or without 0.8 m*M* $CaCl_2$ (free Ca^{2+} 0.3 or 0 m*M*), 8 μg/ml phosphatidylserine and 0.8 μg/ml 1,2-diolein. The reaction was carried out at 30°C for 10 min and terminated by the addition of 2 ml of ice-cold 5%

TCA. After precipitation, materials were collected on a Millipore membrane filter (pore size 0.45 μm; Millipore Products Division; Bedford, MA) and radioactivity was counted by a liquid scintillation counter (LSC-3500E). Activity was expressed by particulate/total (cytosol+particulate) protein kinase C activity (%).

Statistical analysis All values are expressed as the means ±SD (standard deviation). Statistical analysis was carried out using Student's *t* test or analysis of variance (ANOVA).

RESULTS

Effect of IL-1α on ^{3}H-TdR incorporation into NIM 1 cells We have reported that the growth of NIM 1 cells was enhanced by the addition of IL-1α (10 ng/ml).[13] In this study, to find an appropriate concentration of IL-1α for stimulating the proliferation of NIM 1 cells, we studied the dose-response effect of IL-1α on the growth of NIM 1 cells. The ^{3}H-TdR incorporation into DNA of NIM 1 cells was measured. Cell proliferation was tested for 48 h in the presence of various concentrations of IL-1α in the range of 0.001–10 ng/ml. As shown in Fig. 1, IL-1α stimulated the incorporation of ^{3}H-TdR into NIM 1 cells in a dose-dependent manner. The concentration of IL-1α required for significant stimulation was 0.01 ng/ml. The proliferative response of NIM 1 cells reached a peak at the concentration of 1 ng/ml.

Inhibition of IL-1α-stimulated incorporation of ^{3}H-TdR by voltage-dependent Ca^{2+} channel blockers We examined the effect of nicardipine, an inhibitor of voltage-dependent Ca^{2+} channels, on ^{3}H-TdR incorporation stimulated by IL-1α. As shown in Fig. 2, the stimulatory effect of IL-1α was inhibited by nicardipine in a concentration-dependent manner (10–1000 n*M*); the proliferation was decreased to 77, 40 and 16% by 10, 100 and 1000 n*M* nicardipine, respectively. Basal ^{3}H-TdR incorporation of NIM 1 cells was also inhibited by a high concentration of nicardipine (1 μM). The other Ca^{2+} channel blocker, diltiazem, also suppressed the stimulatory effect of IL-1α at concentrations of 0.1–10 μM (data not presented). The effect of Ca^{2+} channel blockers on the growth of NIM 1 cells was not due to a nonspecific cytotoxic effect, because the Ca^{2+} channel blockers used in this study did not affect the viability of NIM 1 cells, and nicardipine is not cytotoxic at concentrations of less than 100 μM.[18]

^{45}Ca influx into NIM 1 cells We examined the effect of IL-1α on ^{45}Ca influx into NIM 1 cells. The influx of

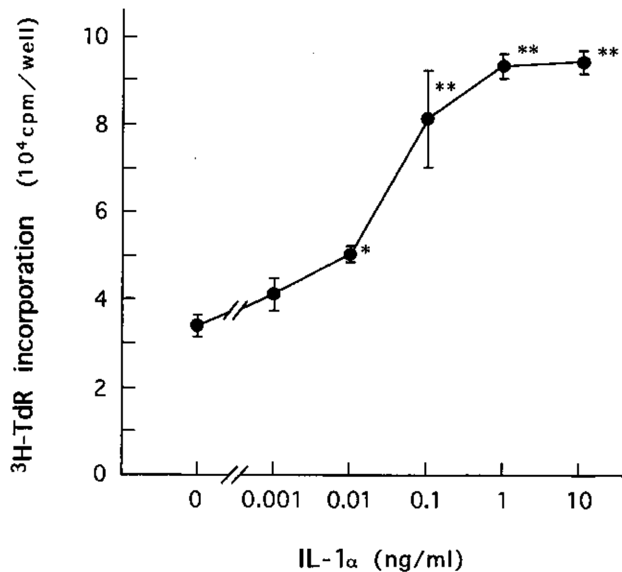

Fig. 1. Effect of IL-1α on ^{3}H-TdR incorporation into NIM 1 cells. NIM 1 cells (1×10^4/well) were cultured for 2 days with various concentrations of IL-1α (0.001–10 ng/ml). After 2 days, cultured cells were pulsed with 0.5 μCi/well of ^{3}H-TdR for 24 h. The radioactivity of incorporated ^{3}H-TdR was measured (see "Materials and Methods"). Data are the means±SD of 3 separate experiments carried out in triplicate and the standard deviations (SD) are indicated by the vertical bars. *, $P<0.05$ vs. control; **, $P<0.01$ vs. control.

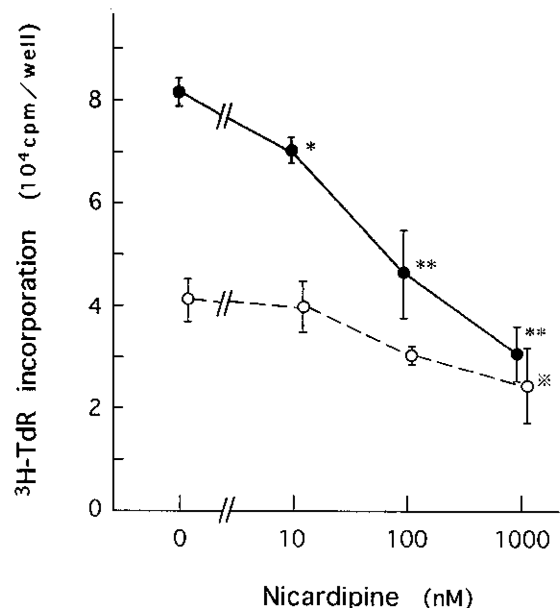

Fig. 2. Inhibitory effect of nicardipine on ^{3}H-TdR incorporation. NIM 1 cells (1×10^4/well) were cultured for 2 days with various concentrations of nicardipine in the presence (●) or absence (○) of IL-1α (1 ng/ml). The incorporation of ^{3}H-TdR into cells was measured. The results are the means ±SD of triplicate cultures. *, $P<0.05$ vs. IL-1α (1 ng/ml); **, $P<0.01$ vs. IL-1α (1 ng/ml); ※, $P<0.01$ vs. control.

$^{45}Ca^{2+}$ during 3 min incubation with $^{45}CaCl_2$ (1.5 μCi) in the presence of various concentrations of IL-1α in the range of 0.01–10 ng/ml was measured. As shown in Fig. 3, IL-1α stimulated the ^{45}Ca influx into NIM 1 cells in a concentration-dependent manner. The maximum response was observed at 1 ng/ml. The stimulatory effect of IL-1α (1 ng/ml) was inhibited by nicardipine in a concentration-dependent manner (100–1000 n*M*), but addition of nicardipine alone resulted in no significant change of basal ^{45}Ca influx (data not presented).

Effect of IL-1α on the level of cyclic AMP in NIM 1 cells To investigate the possibility that IL-1α exerts its biological actions via cyclic AMP, we measured cyclic AMP levels in NIM 1 cells. As shown in Table I, IL-1α did not increase the cyclic AMP in the cells. Forskolin (1 μM), an activator of adenylate cyclase, which was used as a positive control, produced a 7-fold increase in cAMP production.

Effects of protein kinase C inhibitor (H-7) and calmodulin antagonist (W-7) on IL-1α-stimulated incorporation of ^{3}H-TdR There are two routes by which extracellular signals are transduced into intracellular events by the Ca^{2+} messenger system. One is mediated by protein kinase C, which is activated by diacylglycerol or tumor-promoting phorbol esters, at the basal level of Ca^{2+}.[19] The other is mediated by a rise in the cytosolic Ca^{2+} concentration leading to the modulation of calmodulin-dependent reactions. As H-7 inhibits protein kinase C-mediated phosphorylation but not Ca^{2+}/calmodulin-mediated phosphorylation, this agent can serve as a useful tool for clarifying the biological role of the protein kinase C-mediated system.[20] As shown in Fig. 4, the treatment of NIM 1 cells with various concentrations of H-7 (0.1–10 μM) resulted in no significant reduction of ^{3}H-TdR incorporation stimulated by IL-1α (1 ng/ml). It has been documented that an increase in intracel-

Table I. Cyclic AMP Production by Forskolin and IL-1α Treatment in NIM 1 Cells

	Cyclic AMP (pmol/1×10^6 cells)
Control	7.07±0.55
IL-1α (1 ng/ml)	5.00±0.53
Forskolin (1 μM)	50.07±3.50[a]

Cells were preincubated with IBMX (1 m*M*) at 37°C for 10 min and then incubated for another 10 min with or without a test compound (IL-1α or forskolin) in the presence of IBMX (1 m*M*). Cyclic AMP in the cells was measured as described in "Materials and Methods." Data are the means±SD of triplicate assays.
a) Statistically significant difference from the control ($P<0.05$).

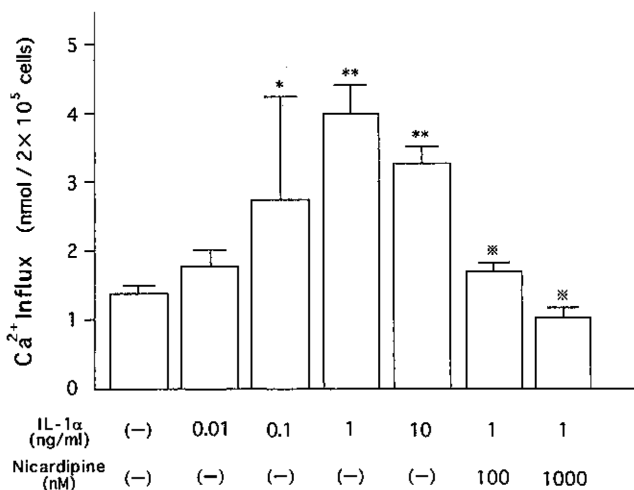

Fig. 3. Effect of nicardipine treatment on basal and IL-1α-stimulated influx of $^{45}Ca^{2+}$. NIM 1 cells were incubated at 37°C for 3 min with $^{45}CaCl_2$ (1.5 μCi) in the absence or presence of IL-1α (0.01–10 ng/ml) and nicardipine (100–1000 n*M*). The influx of $^{45}Ca^{2+}$ into the cells was measured. Data are the means±SD of 3 separate experiments carried out in triplicate. *, $P<0.05$ vs. control; **, $P<0.01$ vs. control; ※, $P<0.01$ vs. IL-1α (1 ng/ml).

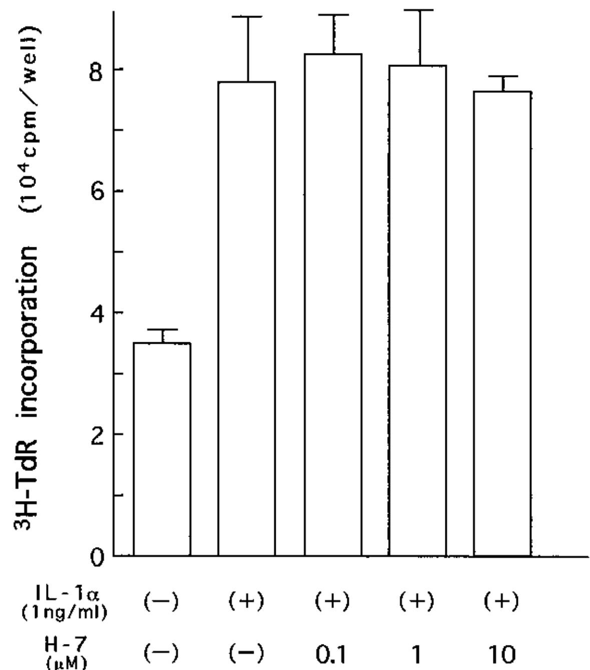

Fig. 4. Effects of various concentrations of H-7 on IL-1α-stimulated incorporation of ^{3}H-TdR. H-7 is a potent and selective inhibitor of protein kinase C. Cells were cultured for 2 days with or without IL-1α (1 ng/ml) and various concentrations of H-7.

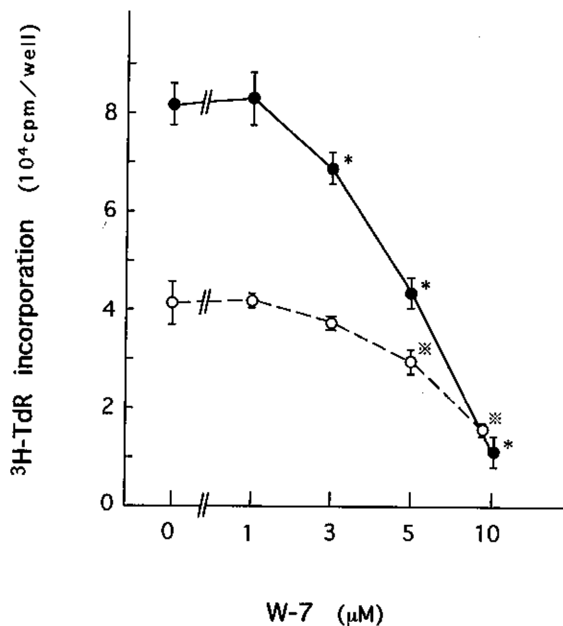

Fig. 5. Inhibitory effect of calmodulin antagonist (W-7) on IL-1α-stimulated incorporation of ^{3}H-TdR. Cells were cultured for 2 days with various concentrations of W-7 in the presence (●) or absence (○) of IL-1α (1 ng/ml). *, $P<0.01$ vs. IL-1α (1 ng/ml); ※, $P<0.01$ vs. control.

lular Ca^{2+} triggers the translocation of protein kinase C to the particulate fraction,[21] where diacylglycerol interacts with protein kinase C to stimulate the enzyme activity.[19, 22] We measured the activity of protein kinase C in cytosol and particulate fractions after stimulation of NIM 1 cells with IL-1α (1 ng/ml) for 5 min at 37°C. IL-1α did not produce any significant change of translocation of protein kinase C from the cytosol to the particulate fraction (basal particulate fraction, 15.0$\pm$4.1% of total protein kinase C activity; IL-1α-stimulated particulate fraction, 18.1$\pm$11.3%).

On the other hand, a calmodulin antagonist such as W-7 binds to calmodulin and selectively inhibits calmodulin-induced enzyme activation.[23] W-7 inhibited the basal and IL-1α-stimulated incorporation of ^{3}H-TdR in a concentration-dependent manner (Fig. 5). The half-maximal and maximal inhibitory concentrations of W-7 were 5 μM and 10 μM for the basal response and also 5 μM and 10 μM for the IL-1α-stimulated response, respectively. W-7 used in this study did not affect the viability of NIM 1 cells.

DISCUSSION

We have demonstrated that the proliferation of NIM 1 cells as determined by measuring ^{3}H-TdR incorporation into NIM 1 cells is enhanced by recombinant IL-1α in a concentration-dependent manner (0.01–1.0 ng/ml). The stimulatory effect of IL-1α on cell proliferation was also reported in other cell lines, such as ATL cells[8] and gastric carcinoma cell lines[9] at concentrations of IL-1α (0.1–1 ng/ml) similar to those used in our study. It was reported that IL-1α exerts its biological effects through an increase in intracellular Ca^{2+} in various cells such as rabbit osteoclasts[24] and rat hepatoma cells.[25] In porcine thyroid cells, epidermal growth factor (EGF) and insulin-like growth factor I (IGF-I), which stimulate thyroid cell proliferation, increase cytoplasmic free calcium, indicating that cytoplasmic free calcium is a possible mediator of thyroid cell proliferation.[26, 27]

In the present study, the effect of IL-1α on cytoplasmic free calcium was studied, because an increase in cytoplasmic free calcium has been implicated as a mediator of mitogenesis in thyroid cells. IL-1α produced an influx of $^{45}Ca^{2+}$ in a concentration-dependent manner similar to that seen in the case of cell proliferation. ^{45}Ca influx occurred within 3 min and thereafter leveled off (data not presented). Furthermore, the stimulatory effects of IL-1α both on cell proliferation and ^{45}Ca influx were inhibited by nicardipine, an inhibitor of L-type voltage-dependent Ca^{2+} channels. The present results, however, do not exclude the possibility of direct effect of nicardipine on cell growth. While the culture medium initially contained 0.75 mM Ca^{2+}, basal ^{3}H-TdR incorporation was inhibited by nicardipine (1000 nM). Also, NIM 1 cells could not be maintained in Ca^{2+}-free culture medium and the cell proliferation was stimulated by the addition of a small amount of $CaCl_2$ to the culture medium (data not presented). These results suggested that IL-1α enhances the proliferation of NIM 1 cells via stimulation of Ca^{2+} influx through voltage-dependent Ca^{2+} channels, and calcium dependency in the growth of NIM 1 cells is essential.

Several lines of evidence have demonstrated that cyclic AMP or diacylglycerol is involved in IL-1α-mediated cellular responses. IL-1α is reported to increase the production of cyclic AMP in human fibroblasts[28] and a human natural killer-like cell line.[29] In NIM 1 cells, forskolin induced cyclic AMP production. However, we could not detect an increase in cyclic AMP in NIM 1 cells stimulated by IL-1α. On the other hand, Rosoff *et al.*[30] reported that IL-1 stimulated the production of diacylglycerol by the hydrolysis of phosphatidylcholine in T lymphocytes. Diacylglycerol is known to be a physiological activator of protein kinase C.[19, 22] To investigate the involvement of protein kinase C in IL-1α-mediated cell proliferation, we examined the effect of H-7, a potent and selective inhibitor of protein kinase C.[20] The stimulatory effect of IL-1α was not significantly inhibited by H-7 at any concentration (0.1–10 μM). Furthermore, IL-

1α had little effect on the translocation of protein kinase C from cytosol to particulate fraction. Therefore, protein kinase C or cyclic AMP may not play an important role in IL-1α-mediated proliferation of NIM 1 cells. Also, we have found that genistein, a tyrosine kinase inhibitor, had no effect on basal and IL-1α-stimulated cell proliferation of NIM 1 cells (data not presented).

Considering the circumstances mentioned above, the stimulation of NIM 1 cell proliferation by IL-1α is probably mediated by an increase in intracellular Ca^{2+}. One of the primary intracellular receptors of Ca^{2+} is calmodulin, a ubiquitous Ca^{2+}-binding protein.[31,32] Ca^{2+}/calmodulin has been reported to have important roles in the regulation of cell proliferation at the early DNA synthesis phase (early S phase) of the cell cycle.[23] In the present study, W-7, an inhibitor of calmodulin, inhibited IL-1α-stimulated cell proliferation, and it is noteworthy that W-7 (5–10 μM) inhibits the basal cell proliferation. Among the targets for Ca^{2+}/calmodulin, Ca^{2+}/calmodulin-dependent protein kinase II is a multifunctional protein kinase that has broad substrate specificities and is implicated in the regulation of cellular Ca^{2+} signals.[33] Ohta *et al.* reported that serum and growth factors elicited the phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II in rat embryo fibroblast 3Y1 cells[34] and that this protein kinase is a dynamic component of the mitotic apparatus, particularly at microtubule-organizing centers during mitosis.[35] On the other hand, the signal transduction pathway of cell proliferation stimulated by growth factors has been extensively investigated as regards its receptor-coupled tyrosine kinase cascades[36,37] and some proto-oncogenes such as c-*fos* and c-*myc*.[38–40] Therefore, further study is needed to see whether there is a cross-talk of these systems with the Ca^{2+}/calmodulin-dependent pathway.

In summary, IL-1α stimulates cell proliferation through the Ca^{2+}/calmodulin-dependent pathway, but not the cyclic AMP or protein kinase C pathway in a human thyroid carcinoma cell line, NIM 1. Further study is required to clarify whether other growth factors which were reported to be produced by NIM 1 cells[12] also affect the cell proliferation in the same manner as IL-1α.

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REFERENCES

1) Oppenheim, J. J., Kovacs, K., Matsushima, K. and Durum, S. K. There is more than one interleukin 1. *Immunol. Today,* **7,** 45–56 (1986).
2) Dinarello, C. A. Interleukin 1. *Rev. Infect. Dis.,* **6,** 51–95 (1984).
3) Schmidt, J. A., Mizel, S. B., Cohen, D. and Green, I. Interleukin 1, a potential regulator of fibroblast proliferation. *J. Immunol.,* **128,** 2177–2182 (1982).
4) Lovett, D. H., Ryan, J. L. and Sterzel, R. B. Stimulation of rat mesangial cell proliferation by macrophage interleukin 1. *J. Immunol.,* **131,** 2830–2836 (1983).
5) Rifas, L., Shen, V., Mitchell, K. and Peck, W. A. Macrophage-derived growth factor for osteoblast-like cells and chondrocytes. *Proc. Natl. Acad. Sci. USA,* **81,** 4558–4562 (1984).
6) Lachman, L. B., Brown, D. C. and Dinarello, C. A. Growth-promoting effect of recombinant interleukin 1 and tumor necrosis factor for a human astrocytoma cell line. *J. Immunol.,* **138,** 2913–2916 (1987).
7) Scala, G., Morrone, G., Tamburrini, M., Alfinito, F., Pastore, C. I., D'Alessio, G. and Venuta, S. Autocrine growth function of human interleukin 1 molecules on ROHA-9, an EBV transformed human B cell line. *J. Immunol.,* **138,** 2527–2534 (1987).
8) Shirakawa, F., Tanaka, Y., Oda, S., Eto, S. and Yamashita, U. Autocrine stimulation of interleukin 1α in the growth of adult T-cell leukemia cells. *Cancer Res.,* **49,** 1143–1147 (1989).
9) Ito, R., Kitadai, Y., Kyo, E., Yokozaki, H., Yasui, W., Yamashita, U., Nikai, H. and Tahara, E. Interleukin 1α acts as an autocrine growth stimulator for human gastric carcinoma cells. *Cancer Res.,* **53,** 4102–4106 (1993).
10) Kimura, H., Yamashita, S., Namba, H., Tominaga, T., Tsuruta, M., Yokoyama, N., Izumi, M. and Nagataki, S. Interleukin-1 inhibits human thyroid carcinoma cell growth. *J. Clin. Endocrinol. Metab.,* **75,** 596–602 (1992).
11) Danforth, D. N., Jr. and Sagagias, M. K. Interleukin-1α and interleukin-6 act additively to inhibit growth of MCF-7 breast cancer cells *in vitro. Cancer Res.,* **53,** 1538–1545 (1993).
12) Tohyama, K., Yoshida, Y., Ohashi, K., Sano, E., Kobayashi, H., Endo, K., Naruto, M. and Nakamura, T. Production of multiple growth factors by a newly established human thyroid carcinoma cell line. *Jpn. J. Cancer Res.,* **83,** 153–158 (1992).
13) Zeki, K., Nakano, Y., Inokuchi, N., Watanabe, K., Morimoto, I., Yamashita, U. and Eto, S. Autocrine stimulation of interleukin-1 in the growth of human thyroid carcinoma cell line NIM 1. *J. Clin. Endocrinol. Metab.,* **76,** 127–133 (1993).
14) Furutani, Y., Notake, M., Yamayoshi, M., Yamagishi, J., Nomura, H., Ohue, M., Furuta, R., Fukui, T., Yamada,

M. and Nakamura, S. Cloning and characterization of the cDNAs for human and rabbit interleukin-1 precursor. *Nucleic Acids Res.*, **13**, 5869–5882 (1985).

15) Kobayashi, H., Endo, K. and Nishimura, K. A case report of a thyroid papillary cancer that manifested leukocytosis and hypercalcemia after radiotherapy for bone metastasis. *Jpn. Cancer Clin.*, **35**, 55–62 (1989).

16) Yanagihara, N., Wada, A. and Izumi, F. Effects of α_2-adrenergic agonists on carbachol-stimulated catecholamine synthesis in cultured bovine adrenal medullary cells. *Biochem. Pharmacol.*, **36**, 3823–3828 (1987).

17) Takai, Y., Kishimoto, A., Kikkawa, U., Mori, T. and Nishizuka, Y. Unsaturated diacylglycerol as a possible messenger for the activation of calcium-activated, phospholipid-dependent protein kinase system. *Biochem. Biophys. Res. Commun.*, **91**, 1218–1224 (1979).

18) Shirakawa, F., Yamashita, U., Oda, S., Chiba, S., Eto, S. and Suzuki, H. Calcium dependency in the growth of adult T-cell leukemia cells *in vitro*. *Cancer Res.*, **46**, 658–661 (1986).

19) Nishizuka, Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science*, **258**, 607–614 (1992).

20) Kawamoto, S. and Hidaka, H. 1-(5-Isoquinolinesulfonyl)-2-methylpiperazine (H-7) is a selective inhibitor of protein kinase C in rabbit platelets. *Biochem. Biophys. Res. Commun.*, **125**, 258–264 (1984).

21) Ho, A. K., Thomas, T. P., Chik, C. L., Anderson, W. B. and Klein, D. C. Protein kinase C: subcellular redistribution by increased Ca^{2+} influx. *J. Biol. Chem.*, **263**, 9292–9297 (1988).

22) Nishizuka, Y. Studies and perspectives of protein kinase C. *Science*, **233**, 305–312 (1986).

23) Hidaka, H., Sasaki, Y., Tanaka, T., Endo, T., Ohno, S., Fujii, Y. and Nagata, T. N-(6-Aminohexyl)-5-chloro-1-naphthalene-sulfonamide, a calmodulin antagonist, inhibits cell proliferation. *Proc. Natl. Acad. Sci. USA*, **78**, 4354–4357 (1981).

24) Yu, H. and Ferrier, J. Interleukin-1 alpha induces a sustained increase in cytosolic free calcium in cultured rabbit osteoclasts. *Biochem. Biophys. Res. Commun.*, **191**, 343–350 (1993).

25) Barke, R. A., Brady, P. S. and Brady, L. J. The Ca^{2+} second messenger system and interleukin-1-alpha modulation of hepatic gene transcription and mitochondrial fat oxidation. *Surgery*, **110**, 285–294 (1991).

26) Takasu, N., Takasu, M., Yamada, T. and Shimizu, Y. Epidermal growth factor (EGF) produces inositol phosphates and increases cytoplasmic free calcium in cultured porcine thyroid cells. *Biochem. Biophys. Res. Commun.*, **151**, 530–534 (1988).

27) Takasu, N., Takasu, M., Komiya, I., Nagasawa, Y., Asawa, T., Shimizu, Y. and Yamada, T. Insulin-like growth factor I (IGF-I) stimulates inositol phosphate accumulation, a rise in cytoplasmic free calcium, and proliferation in cultured porcine thyroid cells. *J. Biol. Chem.*, **264**, 18485–18488 (1989).

28) Zhang, Y., Lin, J. X., Yip, Y. K. and Vilcek, J. Enhancement of cAMP levels and of protein kinase activity by tumor necrosis factor and interleukin 1 in human fibroblasts: role in the induction of interleukin 6. *Proc. Natl. Acad. Sci. USA*, **85**, 6802–6805 (1988).

29) Shirakawa, F., Yamashita, U., Chedid, M. and Mizel, S. B. Cyclic AMP — an intracellular second messenger for interleukin 1. *Proc. Natl. Acad. Sci. USA*, **85**, 8201–8205 (1988).

30) Rosoff, P. M., Savage, N. and Dinarello, C. A. Interleukin-1 stimulates diacylglycerol production in T lymphocytes by a novel mechanism. *Cell*, **54**, 73–81 (1988).

31) Cheung, W. Y. Cyclic 3′,5′-nucleotide phosphodiesterase. *Biochem. Biophys. Res. Commun.*, **38**, 533–538 (1970).

32) Kakiuchi, S., Yamazaki, R. and Nakajima, H. Properties of a heat stable phosphodiesterase activity factor isolated from brain extract. *Proc. Jpn. Acad.*, **46**, 587–592 (1970).

33) Hanson, P. I. and Schulman, H. Neuronal Ca^{2+}/calmodulin-dependent protein kinases. *Annu. Rev. Biochem.*, **61**, 559–601 (1992).

34) Ohta, Y., Ohba, T., Fukunaga, K. and Miyamoto, E. Serum and growth factors rapidly elicit phosphorylation of the Ca^{2+}/calmodulin-dependent protein kinase II in intact quiescent rat 3Y1 cells. *J. Biol. Chem.*, **263**, 11540–11547 (1988).

35) Ohta, Y., Ohba, T. and Miyamoto, E. Ca^{2+}/calmodulin-dependent protein kinase II: localization in the interphase nucleus and the mitotic apparatus of mammalian cells. *Proc. Natl. Acad. Sci. USA*, **87**, 5341–5345 (1990).

36) Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R. and Soltoff, S. Oncogenes and signal transduction. *Cell*, **64**, 281–302 (1991).

37) Ullrich, A. and Schlessinger, J. Signal transduction by receptors with tyrosine kinase activity. *Cell*, **61**, 203–212 (1990).

38) Mine, M., Tramontano, D., Chin, W. W. and Ingbar, S. H. Interleukin-1 stimulates thyroid cell growth and increases the concentration of the c-*myc* proto-oncogene mRNA in thyroid follicular cells in culture. *Endocrinology*, **120**, 1212–1214 (1987).

39) Yamashita, S., Ong, J., Fagin, J. A. and Melmed, S. Expression of the *myc* cellular proto-oncogene in human thyroid tissues. *J. Clin. Endocrinol. Metab.*, **63**, 1170–1173 (1986).

40) Persson, H., Hennighausen, L., Taub, R., Degrado, W. and Leder, P. Antibodies to human c-*myc* oncogene product: evidence of an evolutionarily conserved protein induced during cell proliferation. *Science*, **225**, 687–693 (1984).