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SHORT COMMUNICATION

Inhibition of IL-2 Receptor Induction and IL-2 Production in the Human Leukemic Cell Line Jurkat by a Novel Peptide Inhibitor of Protein Kinase C

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We recently reported that the myristoylated peptide *N*-myristoyl-Lys-Arg-Thr-Leu-Arg (*N*-m-KRTLR) is a novel protein kinase C inhibitor. In this study, we investigated the biological effects of *N*-m-KRTLR using as an *in vitro* model the induction of the IL-2 receptor and IL-2 secretion by Jurkat cells in response to stimulation with 12-*O* tetradecanoylphorbol-13-acetate (TPA) plus phytohemagglutinin (PHA) and TPA plus OKT3 mAb. *N*-m-KRTLR significantly suppressed induction of the IL-2 receptor on the surface of the Jurkat cells by TPA plus either PHA or OKT3 mAb. Furthermore, *N*-m-KRTLR inhibited the production and release of IL-2 from cultured Jurkat cells stimulated with TPA plus either PHA or OKT3 mAb. Similarly, this peptide significantly inhibited the IL-2 production in normal human peripheral blood mononuclear cells in response to stimulation by TPA and PHA. In contrast, this peptide did not affect expression of the CD3 complex on the surface of the Jurkat cells either alone or in the presence of TPA or PHA. Furthermore, *N*-m-KRTLR did not interfere with the spontaneous proliferation of the Jurkat cells, and its effects on IL-2 secretion and IL-2 receptor expression in the Jurkat cells were evident without loss of cell viability. These results suggest that the novel protein kinase C inhibitor *N*-m-KRTLR may selectively inhibit certain activation pathways of Jurkat cells and indicate the usefulness of *N*-m-KRTLR in the analysis of discrete events in T cell activation.

INTRODUCTION

Stimulation of T lymphocytes with anti-CD3 antibodies or with mitogenic lectins either alone or in combination with phorbol-ester tumor promoters causes T cell activation (1–3). Activation of T lymphocytes results in induction of the interleukin-2 receptor (IL2R) and, in certain cells, secretion of interleukin 2 (IL-2) (4, 5). Upon binding to its receptor, IL-2 stimulates the proliferation of activated T lymphocytes (2). Recent studies provide evidence for a central role for protein kinase C (PKC) in T lymphocyte activation (6, 7). Protein kinase C (PKC) is comprised of a family of

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Ca^{2+}- and phospholipid-dependent protein kinases that are stimulated by diacylglycerol, a second messenger of signal transduction pathways that involve polyphosphoinositide hydrolysis (8–10).

N-myristoyl-Lys-Arg-Thr-Leu-Arg (*N*-m-KRTLR) is a novel PKC inhibitor (inhibitory constant, IC_{50} = 75 μM) insofar as it binds to the phosphoacceptor substrate binding site of PKC and interferes with the activation of the enzyme by allosteric cofactors (11). The binding to the phosphoacceptor binding site of PKC by *N*-m-KRTLR suggests that the peptide interacts selectively with the active site of PKC, and this is supported by the observation that *N*-m-KRTLR is a relatively weak inhibitor of the closely related enzyme, cAMP-dependent protein kinase (IC_{50} > 400 μM) (11). In this study, we demonstrate that *N*-m-KRTLR antagonizes early events in the activation of T lymphocytes by phorbol ester tumor promoters, the OKT3 mAb, PHA, and various combinations of these mitogens.

The T lymphoblastoid cell line Jurkat expresses the IL-2R and produces large amounts of IL-2 when activated by PHA or OKT3 mAb in the presence of TPA. Thus, the analysis of IL-2R expression provides a useful model for investigating the effects of various PKC activators and inhibitors on T cell activation.

MATERIALS AND METHODS

Monoclonal Antibodies and Chemicals

OKT3 fluorescein isothiocyanate (FITC)-conjugated OKT3 mAb was obtained from Ortho Diagnostic (Raritan, NJ). Anti-IL-2R mAb (anti-Tac) phycoerythrein-conjugated (a-IL-2R-PE) was obtained from Becton-Dickinson (Mountain View, CA). FITC-conjugated F(ab)2 fragments of goat anti-mouse were purchased from Boehringer-Mannheim Biochemicals (Indianapolis, IN). TPA and the PKC inhibitors H-7 and HA-1004 were purchased from Sigma Chemical Co. (St. Louis, MO). TPA was stored at 1 mg/ml in DMSO at −20°C.

N-m-KRTLR was synthesized and purified as we have previously described (11).

Cell Cultures

Jurkat cells (T lymphoblastoid) were cultured in RPMI 1640 medium with 10% FCS and 40 ng/ml gentamicin (complete RPMI 1640 medium). Peripheral blood mononuclear cells (PBMC) were isolated from normal volunteers by Ficoll–Hypaque (Hystopaque 1077-Sigma) gradient centrifugation. Cells from interface were collected, washed three times, and suspended in culture at a final concentration of 1×10^6 cells/ml and stimulated with 10 μg/ml PHA, in the presence or absence of PKC activators (TPA) and inhibitors (*N*-m-KRTLR) as described below. The cells were maintained at 37°C in a humidified atmosphere of 5% CO2. Jurkat cells (0.5×10^6/ml) were cultured with TPA (0.5 and 5.0 ng/ml) in the presence or absence of either OKT3 mAb (1/250 dilution of ascites) or 1 μg/ml PHA. Peptide inhibitors were added at indicated concentrations at the beginning of the cultures. The PBMC and Jurkat cells were incubated for 24 hr with these inhibitors before the cells and supernatants were collected and employed for quantitation of IL-2R expression and IL-2 production.

Cell Surface Immunofluorescence

To determine the IL-2R and CD3 antigen expression, Jurkat cells were stained with the anti-Tac-PE and OKT3-FITC mAb and the expression of both antigens was de-

termined as we have previously described (13). In brief, Jurkat cells were washed free of medium and incubated with anti-Tac-PE and OKT3-FITC mAb in Hanks' balanced salt solution containing 0.1% NaN3 for 30 min at 4°C and further washed before being examined by flow cytometry (in FACS-Star, Becton-Dickinson) as previously described (13, 14). Results are expressed as the percentage positive cells (see Fig. 1).

Determination of IL-2 Synthesis

The IL-2 dependent murine natural killer cell line NK-8 (15) was used to measure IL-2 activity, as previously described (14). In brief, 10^4 NK-8 cells were cultured with various dilutions of IL-2-containing supernatants. Afterward 1 μCi of tritiated thymidine TdR was added to the wells for the last 8 hr of incubation. The cells were harvested on a cell harvester (Brandel, Gaithersburg, MD). [^{3}H]TdR incorporation was counted in a Beckman LS3801 scintillation counter (Beckman Instruments, Fullerton, CA). The concentrations of IL-2 in the culture supernatants were calculated at multiple concentrations by dilution analysis of each supernatant, compared with recombinant IL-2 (Cetus), and expressed in units/ml medium as described (16, 17).

RESULTS

Inhibition of IL-2R Induction in Jurkat Cells by N-m-KRTLR

The novel PKC inhibitor *N*-m-KRTLR (IC_{50} = 75 μM) (11) was used to examine the effects of PKC inhibition on the induction of the cell surface expression of IL-2R by TPA plus either OKT3 mAb or PHA. The phenotype of Jurkat cells employed in these experiments was CD3+, CD2+, CD4+, CD8−, CD45R+, and CD25−, 60% TdT+, HLA−DR+, CD5+. To investigate the effects of this peptide on the induction of IL-2R and CD3 antigen expression, Jurkat cells were incubated for 24 hr with TPA, OKT3 mAb, PHA, or a combination of TPA plus either PHA or OKT3. Two representative experiments are presented in Tables 1 and 2. Induction of IL-2R by the OKT3 mAb alone was minimal (Table 1). Significant induction of IL-2R was achieved by 5.0 ng/ml TPA and by a combination of TPA and OKT3 mAb (Tables 1 and 2). *N*-m-KRTLR significantly inhibited induction of IL-2R by TPA at 5.0 ng/ml and inhibited induction by the combination of OKT3 mAb and 5.0 ng/ml TPA (Tables 1 and 2). However, the observed inhibition by *N*-m-KRTLR was more pronounced with a combination of TPA and OKT3 mAb than with TPA alone. Significant inhibition of IL-2R induction was also achieved by the peptide when Jurkat cells were stimulated by a combination of PHA and TPA (Table 2). Under these experimental conditions, the down-modulation of the CD3 antigen was minimal, and it was not significantly affected by the presence of *N*-m-KRTLR (Tables 1, 2). In further experiments, TPA at 50 ng/ml was employed for 1 hr to induce significant down-modulation of CD3 antigen on Jurkat cells. Similarly, *N*-m-KRTLR did not affect the down-modulation of the CD3 antigen under these conditions. The expression of CD3 antigen was affected by TPA (86.5% CD3-positive cells in control cultures versus 44.2% CD3 positive cells in cultures treated with TPA). However, this down-modulation was not inhibited by *N*-m-KRTLR (44.5% CD3-positive cells in the presence of TPA plus *N*-m-KRTLR).

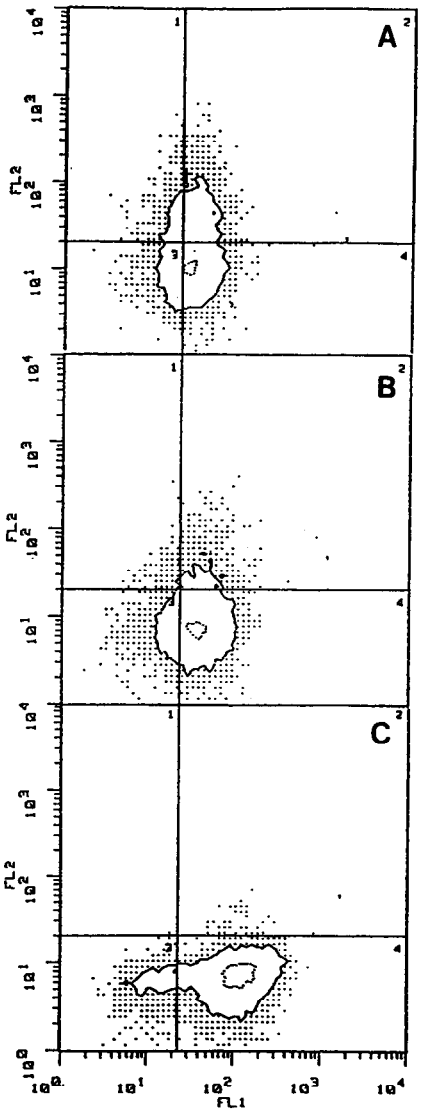

FIG. 1. Two-color FACS analysis of the expression of the IL-2R (red, x axis) and CD3 antigen (green, y axis) on Jurkat cells. (A) Jurkat cells stimulated with TPA (5.0 ng/ml) and OKT3 mAb (1/250 dilution); (B) Jurkat cells stimulated with TPA (5.0 ng/ml) and OKT3 mAb (1/250) in the presence of myristoylated peptide, and stained with OKT3-FITC and anti-Tac-PE mAb; (C) OKT3-FITC plus anti-Tac-PE conjugated on Jurkat cells.

To assess the efficacy of *N*-m-KRTLR as an inhibitor of IL-2R induction in Jurkat cells, we compared its inhibitory activity to that of the isoquinolinesulfonamide protein kinase inhibitor H-7. The isoquinolinesulfonamides H-7 and HA-1004 are cell-permeable protein kinase inhibitors that compete with the substrate ATP at protein kinase active sites. Since H-7 inhibits PKC and cyclic nucleotide-dependent protein kinases with similar potencies, and since HA-1004 inhibits cyclic nucleotide-dependent protein

TABLE 1

Inhibition of the Induction of the IL-2R by TPA and OKT3 mAb on Jurkat Cells by the Peptide *N*-m-KRTLR[a]

Inducer	*N*-m-KRTLR (μM)	% Positive cells: CD3 (Green fluorescence)	% Positive cells: IL-2R (Red fluorescence)	Inhibition[b] (%)
A. Medium +				
1.	0	90.38	0.46	—
B. OKT3 mAb +				
1.	0	87.4	1.6	
2.	50	86.0	0.4	
3.	25	86.7	0.4	
C. TPA 5.0 ng/ml				
1.	0	87.7	32.0	—
2.	50	89.2	20.9	35
3.	25	91.7	23.1	28
D. TPA (0.5 ng/ml) + OKT3 mAb				
1.	0	91.6	3.4	—
2.	50	89.6	0.5	86
3.	25	88.8	2.2	36
E. TPA (5.0 ng/ml) + OKT3 mAb				
1.	0	69.7	34.0	—
2.	50	80.8	9.9	71
3.	25	79.8	12.8	62

[a] Jurkat cells were cultured with the above indicated concentrations of TPA and OKT3 mAb (1/250 dilution of ascites), washed three times in HBSS, and further stained with antibodies as described under Materials and Methods. Ten thousand Jurkat cells were examined for the expression of IL-2R and CD3 antigens.

[b] Percentage of inhibition was calculated by considering the corresponding sample that received inducers only as 100%.

kinases potently but inhibits PKC weakly (18), biological effects observed with H-7 but not with HA-1004 have been attributed to inhibition of PKC. We also found that induction of IL-2R on Jurkat cells mediated by a combination of TPA and PHA or by TPA alone was inhibited by H-7 but not by HA-1004 (Table 3). H-7 and *N*-m-KRTLR inhibited IL-2R expression with comparable efficacies, despite the fact that they inhibit isolated PKC by different mechanisms.

Effects of N-m-KRTLR on the Proliferation of Jurkat Cells

To investigate the specificity of the effects of *N*-m-KRTLR on IL-2R expression on Jurkat cells, we examined its effects on both cellular viability and proliferation. No loss of cellular viability was observed when the Jurkat cells were incubated with *N*-m-KRTLR at concentrations as high as 50 μM. The viability of the cultured cells incubated

TABLE 2

Inhibition of the Induction of the IL-2R by TPA and PHA on Jurkat Cells by the Peptide *N*-m-KRTLR[a]

Inducer	*N*-m-KRTLR (μM)	% Positive cells: CD3 (Green fluorescence)	% Positive cells: IL-2R (Red fluorescence)	Inhibition (%)
A. Medium +				
1.	0	90.5	0.3	
2.	50	88.8	1.7	
3.	25	90.8	1.9	
B. TPA (0.5 ng/ml) +				
1.	0	90.8	13.2	—
2.	50	92.0	4.6	65
3.	25	93.1	5.5	58
C. TPA (5.0 ng/ml) +				
1.	0	92.6	18.2	—
2.	50	83.8	6.7	63
3.	25	88.0	8.7	52
D. PHA +				
1.	0	87.6	18.6	—
2.	50	90.8	14.4	23
3.	25	90.4	15.3	18
E. PHA + 0.5 ng/ml TPA +				
1.	0	79.0	27.8	—
2.	50	83.7	18.5	34
3.	25	90.4	21.2	24
F. PHA + 5.0 ng/ml TPA +				
1.	0	76.5	26.8	—
2.	50	83.1	13.5	53
3.	25	87.6	17.7	59

[a] PHA was employed at 1 μg/ml final concentration. For further details see legend to Table 1.

with *N*-m-KRTLR for 24 hr ranged from 95 to 98%, providing evidence that *N*-m-KRTLR did not have direct toxic effects on the Jurkat cells. Furthermore, *N*-m-KRTLR did not affect the spontaneous proliferation of the Jurkat cells. After Jurkat cells (50,000 cells/ml) were incubated with 50 μM peptide for 24 hr, the number of viable cells observed (92,000 $\pm$ 3500) was 88 $\pm$ 3% of the cell number observed after a 24 hr incubation of the cells with medium alone. These results were confirmed by measuring the incorporation of [^{3}H]TdR in Jurkat cell cultures in the presence or absence of *N*-m-KRTLR (Table 4). *N*-m-KRTLR by itself did not inhibit the [^{3}H]TdR incorporation in Jurkat cells. Significant inhibition of the proliferation of Jurkat cells has been observed with TPA. We found that the peptide did not affect TPA-induced inhibition of the proliferation of Jurkat cells (Table 4).

Inhibition by N-m-KRTLR of IL-2 Secretion by Jurkat Cells

In addition to IL-2R induction, IL-2 secretion correlates with T cell activation. IL-2 secretion by Jurkat cells was stimulated by PHA or by a combination of PHA and

TABLE 3

Inhibition of the Induction of IL-2R on Jurkat Cells by H-7 and HA1004[a,b]

Inducer	% Positive cells		Inhibition (%)
	CD3	IL-2R	
A. Medium	90.38	0.46	
B. TPA	87.76	32.04	
1. TPA + HA 1004 (10 μM)	89.45	30.54	5
2. TPA + HA 1004 (1 μM)	90.56	31.49	—
3. TPA + H7 (10 μM)	89.47	22.45	43
4. TPA + H7 (1 μM)	90.51	29.77	7
C. TPA + PHA	69.75	33.99	
1. TPA + PHA + H7 (10 μM)	89.75	23.25	32
2. TPA + PHA + H7 (1 μM)	87.56	29.85	12

[a] TPA (5 ng/ml) and PHA (1 μg/ml) were used for the induction of the IL-2R on Jurkat cells.
[b] The inhibitors H-7 and HA-1004 were used at the concentrations indicated in the table.

TPA. Jurkat cells did not constitutively secrete IL-2, and furthermore, they did not secrete IL-2 when stimulated by phorbol esters alone. We investigated the effects of *N*-m-KRTLR on the secretion of IL-2 by Jurkat cells. A representative experiment is presented in Table 5. Significant inhibition of IL-2 release in response to PHA alone and to PHA plus TPA was observed when Jurkat cells were incubated with 50 μM peptide, and the inhibitory activity of the peptide was dose dependent. Thus, according to these two parameters, (IL-2R expression and IL-2 secretion) *N*-m-KRTLR antagonized activation of Jurkat cells by TPA alone, and by TPA plus OKT3 mAb and/or PHA.

TABLE 4

Effect of the Peptide *N*-m-KRTLR on the Proliferation of Jurkat cells[a]

Stimulus	*N*-m-KRTLR (μM)	[^{3}H]TdR incorporation (cpm)	
		Experiment 1	Experiment 2
A. Medium	0	65,370	78,450
TPA (0.5 ng/ml)	0	30,440	38,575
TPA (5.0 ng/ml)	0	15,916	18,275
Medium	50	70,193	81,256
B. TPA (0.5 ng/ml)	25	32,500	43,648
TPA (0.5 ng/ml)	50	37,853	45,375
C. TPA (5.0 ng/ml)	25	19,756	23,119
TPA (5.0 ng/ml)	50	19,678	24,579

[a] Jurkat cells (5×10^4) were cultured with the concentrations of the inducer indicated above, for 20 hr at 37°C in 96-well flat-bottomed tissue culture plates in complete RPMI 1640 medium. Afterward, 1 μCi of [^{3}H]TdR was added to each well, and the cells were harvested 16 hr later.

TABLE 5

Inhibition of IL-2 Production of Jurkat Cells Induced by TPA and PHA by the Peptide *N*-m-KRTLR[a]

Stimulus	*N*-m-KRTLR[b] (μM)	[^{3}H]TDR incorporation (cpm)	Inhibition (%)
A.			
1. Medium	0	800	
2. TPA (0.5 ng/ml)	0	1,215	
3. TPA (5.0 ng/ml)	0	1,000	
B. PHA			
1. PHA	0	4,899	—
2. PHA	12	4,513	8
3. PHA	25	3,431	30
4. PHA	50	2,241	54
C. PHA + 0.5 ng/ml TPA			
1.	0	14,809	—
2.	12	16,848	0
3.	25	9,080	39
4.	50	4,925	67
D. PHA + 5.0 ng/ml TPA			
1.	0	14,040	—
2.	12	15,661	0
3.	25	8,663	38
4.	50	8,480	40

[a] 100 μl of supernatant was removed from each well and assessed for the ability to support the proliferation of the IL-2-dependent murine NK-8 cell line.

[b] Jurkat cells were cultured alone or with TPA and/or PHA for 24 hr with the indicated concentrations of peptide.

Inhibition by N-m-KRTLR of IL-2 Secretion by PBMC

To investigate the effects of the PKC inhibitor *N*-m-KRTLR in the IL-2 secretion by PBMC, we studied the effects of this inhibitor on the IL-2 production in response to PHA and the phorbol ester TPA (Table 6). *N*-m-KRTLR significantly inhibited the IL-2 secretion induced by stimulation with PHA plus TPA in PBMC. This inhibition was peptide concentration dependent. At 50 μM peptide the IL-2 secretion was inhibited by 88–90% when 5 ng/ml TPA was used, whereas at 0.5 ng/ml TPA the levels of IL-2 measured were not significantly different from the SEM.

DISCUSSION

In this report we demonstrate that a novel PKC inhibitor, *N*-m-KRTLR, (11) inhibits IL-2 secretion and IL-2 receptor induction by TPA plus either PHA or OKT3 mAb in a well-characterized T lymphoblastoid cell line (Jurkat). These inhibitory effects were observed without a concomitant decrease in cell proliferation or cell viability.

Previously, we reported that *N*-myristoylation of the PKC substrate KRTLR (Km app = 300 ± 40 μM) endows the peptide with an inhibitory activity against isolated PKC (IC_{50} = 75 μM) without improving its parameters as a PKC substrate (11, 12). Kinetic studies provided evidence that *N*-m-KRTLR is a novel PKC inhibitor, since it both antagonized activation of PKC by allosteric cofactors and competed with poly-

TABLE 6

Inhibition of IL-2 Production by PBMC Induced by PHA and TPA by the Peptide *N*-m-KRTLR[a]

Stimulus (μM)	*N*-m-KRTLR	IL-2 (U/ml medium) Donor 1	Donor 2
A.			
Medium	0	0	0
TPA (0.5 ng/ml)	0	<1	<1
TPA (5.0 ng/ml)	0	<1	<1
PHA	0	22 ± 5	34 ± 7
B. PHA + 0.5 ng/ml TPA			
1.	0	38 ± 5	51 ± 3
2.	12	23 ± 5	36 ± 4
3.	25	12 ± 3	16 ± 3
4.	50	<5	<5
C. PHA + 5.0 ng/ml TPA			
1.	0	119 ± 6	137 ± 8
2.	12	79 ± 8	98 ± 12
3.	25	48 ± 7	54 ± 4
4.	50	12 ± 4	16 ± 5

[a] PBMC (1×10^6) from two normal volunteers were cultured alone or with the indicated concentrations of TPA and 10 μg/ml PHA for 24 hr in complete RPMI 1640 medium. The PKC inhibitor peptide *N*-m-KRTLR was added in the cultures at the concentrations indicated in the table. Afterward, supernatants were collected and tested for the ability to support the proliferation of the IL-2-dependent NK-8 murine NK clone. NK-8 cell cultures which contained 1×10^4 indicator cells and binary dilutions of the PBMC supernatant were incubated for 16 hr, and pulsed with 1 μCi of [^{3}H]TdR for 8 additional hr. The units of IL-2 determined for each stimulant and inhibitor are means of three experiments ± SEM.

peptide substrates at the active site of the enzyme (11). The possibility that *N*-m-KRTLR could inhibit PKC activity in intact cells is suggested by the fact that *N*-m-KRTLR is a highly amphiphilic molecule composed of a hydrophobic fatty acid chain attached to a basic pentapeptide. The hydrophobic chain of *N*-m-KRTLR presumably could facilitate the insertion of the peptide moiety into the lipid bilayer of the cell membrane. Since PKC is involved in pathways of T cell activation through its signal transducing function, we chose this system to determine whether *N*-m-KRTLR had any biological effects in the cellular environment, in addition to its demonstrated ability to inhibit PKC in a noncellular system. We studied the Jurkat cell line, because it is well-known that activation of PKC by TPA is essential for IL-2R induction in these cells.

From the results presented in this report, it is clear that *N*-m-KRTLR can inhibit induction of IL-2R on Jurkat cells by the PKC activator TPA. In addition, when Jurkat cells were incubated with either PHA or OKT3 mAb in addition to TPA, *N*-m-KRTLR inhibited IL-2R induction to a similar extent. In contrast, when Jurkat cells were stimulated with PHA alone, significantly less inhibition of IL-2R induction by *N*-m-KRTLR was observed. Moreover, when Jurkat cells were stimulated by the OKT3 mAb alone, the induction of the IL-2R was minimal, although significant inhibition of the induction was observed with the peptide.

Our results also demonstrated that *N*-m-KRTLR is capable of inhibiting the IL-2 secretion by normal resting T cells within the PBMC when stimulated by PHA and

phorbol ester. Therefore, it is possible that *N*-m-KRTLR interferes with the activation of Jurkat cells and normal resting T cells by similar mechanisms. Furthermore, in preliminary experiments, we have observed that *N*-m-KRTLR inhibited the blastogenesis and proliferation of PBMC from normal donors stimulated in culture with OKT3 mAb (C. G. Ioannides and C. A. O'Brian, manuscript in preparation). It was previously reported that although either OKT3 mAb or PHA alone can activate PKC in T lymphocytes, the effects of TPA are the most effective and of the longest duration. Our results appear to reflect differences in the abilities of the inducers employed in these experiments to activate PKC in Jurkat cells. The ability of *N*-m-KRTLR to inhibit induction of IL-2R in Jurkat cells was comparable to that of the well-known potent PKC inhibitor H-7 (16). Both agents inhibited the secretion of IL-2 from Jurkat cells induced by TPA plus PHA. Secretion of IL-2 is not totally dependent on TPA; TPA alone has been described as ineffective in inducing IL-2 secretion (6). However, the combination of TPA with either mitogenic lectins or anti-CD3 mAb renders Jurkat cells capable of IL-2 secretion (16). Thus, OKT3 mAb and PHA may employ signal transduction pathways that do not merely involve production of diacyglycerol, the endogenous analog of TPA. Since IL-2 is known to up-regulate its receptor, inhibition of IL-2 secretion by *N*-m-KRTLR could amplify the inhibitory effects of *N*-m-KRTLR on IL-2R expression.

N-m-KRTLR and H-7 inhibit PKC by different mechanisms. H-7 competes with ATP at the ATP binding site of the enzyme (18), whereas *N*-m-KRTLR competes with the polypeptide substrate and also antagonizes activation of PKC by Ca^{2+}, TPA, and phospholipids (11, 19, 20). Recent evidence has proved the existence of at least seven PKC isozymes with subtle differences in their modes of activation. Furthermore, PKC isozymes have closely related but distinct polypeptide substrate specificities (10). Therefore, PKC inhibitors that compete with polypeptide substrates of the enzyme, such as *N*-m-KRTLR, have greater potential than ATP antagonists such as H-7 (which inhibits at least several different protein kinases) (18) as prototypes for the design of more selective inhibitors of PKC isoenzymes. Since activation of T lymphocytes by antigens requires binding of the antigen to the TCR followed by PKC-catalyzed phosphorylation of the CD3 complex, selective PKC inhibitors may antagonize T cell activation with high specificity. Our present results demonstrate that the novel PKC inhibitor *N*-m-KRTLR inhibits cellular processes associated with the early events of T cell activation. The use of *N*-m-KRTLR as a model peptide for the design of more selective inhibitors of PKC or of specific PKC isozymes could result in the development of PKC inhibitors that specifically affect critical signal-transduction pathways in immunoregulation.

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