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Regulatory roles of adenylate cyclase and cyclic nucleotide phosphodiesterases 1 and 4 in interleukin-13 production by activated human T cells

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Abstract

We studied the activities of 3′,5′-adenosine-cyclic monophosphate (cAMP)- synthesizing adenylate cyclase (AC) and cAMP-hydrolyzing cyclic nucleotide phosphodiesterase (PDE) in phytohemagglutinin (PHA)- or anti-CD3 plus anti-CD28-stimulated human T cells, and examined their roles in interleukin-13 (IL-13) production. The AC inhibitor MDL 12,330A [*cis-N*-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine hydrochloride] completely blocked PHA- or anti-CD3/CD28-induced IL-13 production. The PDE 1 inhibitor 8-methoxymethyl-3-isobutyl-1-methylxanthine or the PDE4 inhibitor rolipram partially inhibited IL-13 production, and the addition of both resulted in 100 or 85% inhibition in PHA- or anti-CD3/CD28-stimulated T cells, respectively. AC in T cells was transiently activated 5 min after stimuli, followed by the transient activation of PDE4 at 30 min. PDE1 activity, undetectable in resting T cells, was detected 3 hr after stimuli, and then increased gradually. Although PDE1-, 2-, 3-, and 4-independent PDE activity was low (≤15% of total), it began to increase 3 hr after anti-CD3/CD28; the increase was blocked by PDE7 antisense oligonucleotide, and such an increase was not induced by PHA. PHA or anti-CD3/CD28 induced PDE1B mRNA expression, undetectable in resting T cells. PDE4 mRNA level in T cells was not altered by either stimulus. PDE7 mRNA expression was detected in resting T cells, and was enhanced by anti-CD3/CD28, but not by PHA. The cAMP level of T cells increased 5 min after stimuli, returned to the basal level at 2 hr, and then continued to decrease. These results suggest that PHA or anti-CD3/CD28 initially (≤5 min) increases cAMP in T cells via AC, then reverses the increase via PDE4 (≤2 hr), and in the later phase (>2 hr) further decreases cAMP via PDE1. Both the time-dependent increase and decrease of cAMP may be required for IL-13 production.

Keywords: Phytohemagglutinin; Anti-CD3; Anti-CD28; Interleukin-13; Adenylate cyclase; Cyclic nucleotide phosphodiesterase

1. Introduction

IL-13 is a B cell-stimulatory cytokine that induces IgE and IgG4 production in B cells by isotype switching [1], and may be involved in the pathogenesis of certain allergic diseases such as atopic dermatitis or asthma [2,3]. This cytokine is produced mainly by T cells [1], and the production is induced by PHA or anti-CD3 plus anti-CD28 antibodies [4,5]. However, signaling pathways involved in IL-13 production have not been identified definitively. It was reported recently that cAMP-elevating agents inhibit allergen-induced IL-13 production in human T cells and basophils [6,7]. It also has been reported that prolonged and excessive cAMP signal down-regulates the other T cell functions such as proliferation, IL-2 or IL-4 production, or IL-2 receptor expression [6,8–10]. Cellular cAMP levels are controlled by AC, which synthesizes cAMP, and by PDE, which hydrolyzes cAMP [11]. Thus, cAMP accumulation is manifested by AC-stimulating agents, such as prostaglandin E_2 or forskolin [8], and by PDE inhibitors, such as theophylline [6].

Previous studies especially support the requirement of

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Abbreviations: AC, adenylate cyclase; AP, activator protein; AS-O, antisense oligonucleotide; cAMP, 3′,5′-adenosine-cyclic monophosphate; cGMP, 3′,5′-guanosine-cyclic monophosphate; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine; GAM, goat anti-mouse IgG polyclonal antibody; IBMX, 3-isobutyl-1-methylxanthine; IL-13, interleukin-13; 8-methoxymethyl-IBMX, 8-methoxymethyl-3-isobutyl-1-methylxanthine; NS-O, nonsense oligonucleotide; PCR, polymerase chain reaction; PDE, cyclic nucleotide phosphodiesterase; PHA, phytohemagglutinin; RT, reverse transcription; and TCR, T cell receptor.

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PDE for IL-13 production [6,7]. PDE may possibly reduce the cellular cAMP level and thus prevent the inhibitory effect of cAMP accumulation on IL-13 production. PDE is an isozymic family composed of various subtypes classified on the basis of substrate specificity, inhibitor specificity, and sequence homology [7]. The previous paper suggests that cAMP-specific PDE (PDE4) may be involved in IL-13 production by human T cells [7].

In this study, we investigated the involvement of AC and PDE in PHA- or anti-CD3 plus anti-CD28-induced IL-13 production of T cells. We found that AC and PDE activities were time-dependently up- and down-regulated after the stimuli, and that both were required for IL-13 production.

2. Materials and methods

2.1. Reagents

The purified form of *Phaseolus vulgaris* PHA, actinomycin D, cycloheximide, EHNA, and affinity-purified GAM were purchased from the Sigma Chemical Co. Rolipram, 8-methoxymethyl-IBMX, cilostamide, MDL 12,330A [*cis*-*N*-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine hydrochloride], SQ22536 [9-(tetrahydro-2′-furyl)adenine], 2′,5′-dideoxyadenosine, vinpocetine, Ro-20–1724 [4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone], milrinone, IBMX, 8-bromo-cAMP, and H-89 [*N*-[2-((*p*-bromocinnamyl)amino)ethyl]-5-isoquinolinesulfonamide] were obtained from Calbiochem, and were dissolved in dimethyl sulfoxide as 10 mM stock solutions and kept in the dark until used. Anti-CD3 monoclonal antibody (OKT3, murine IgG2a) and anti-CD28 monoclonal antibody (clone 9.3, murine IgG2a) were purchased from Becton Dickinson.

2.2. Preparation of human T cells

Blood was taken from five healthy Japanese volunteers [two men and three women, age 43.6 ± 13.6 years (mean ± SD)], who were informed of the objectives and methods of this study, and consented to participate. Peripheral blood mononuclear cells were isolated by centrifugation over Ficoll-Paque (Pharmacia) as described [12], and were allowed to adhere to plastic dishes for 1 hr at 37°. From the non-adherent cells, $CD56^-$ cells were isolated by negative selection using immunomagnetic beads (Dynal) as described [13], and were incubated with neuraminidase-treated sheep erythrocytes as described [14]. From the rosette-forming cells, $CD14^-$ and $CD19^-$ cells were isolated by immunomagnetic negative selection, and were used as T cells. This T cell population was >98% $CD3^+$, and the contamination of $CD14^+$, $CD19^+$, or $CD56^+$ cells was <2%.

2.3. Measurement of IL-13 and cAMP

T cells (2×10^5/200 μL/well) were cultured with 10 μg/mL of PHA or anti-CD3 plus anti-CD28 antibodies (each 0.1 μg/mL) in plates coated with GAM (10 μg/mL) as described [15], at 37° in an atmosphere of 5% CO_2 for 48 hr. T cells were cultured with medium alone in parallel. Each culture was performed in triplicate. The culture medium used was a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's Nutrient Mixture F-12 (Sigma), supplemented with 2.5 mM L-glutamine (Gibco/BRL). The activity of IL-13 in the culture supernatants was measured by an ELISA kit (Biosource). The sensitivity of the assay was 12 pg/mL. To analyze the inhibitory effects of various AC or PDE inhibitors on PHA- or anti-CD3/CD28-induced IL-13 production, these agents were added at various time points before, simultaneously, or after the stimuli. The percent inhibition of either stimulus-induced IL-13 production was calculated by the following equation: (IL-13 amount with stimulus − IL-13 amount with stimulus plus inhibitor) ÷ (IL-13 amount with stimulus − IL-13 amount with medium alone) × 100 (%). To measure the intracellular cAMP level at various time points, medium was discarded and cells were lysed with ethanol, the lysates were centrifuged at 10,000 *g* for 10 min at 4°, and the supernatants were dried under vacuum. The dried samples were dissolved in acetate buffer (pH 5.8), and cellular cAMP contents were measured by an ELISA kit from Amersham. The sensitivity of the assay was 12 fmol/assay well. The cAMP level was presented as picomoles per 10^6 cells.

2.4. Measurement of PDE activity

T cells were lysed in a buffer containing 20 mM Tris–HCl (pH 7.4), 1 mM EDTA, 1 μg/mL of aprotinin, 1 μg/mL of pepstatin, 1 μg/mL of leupeptin, 15 mM benzamidine, and 3.75 mM β-mercaptoethanol. PDE activity of the cell lysates was assayed as described previously [8,16] using 1 μM [2,8-^{3}H]cAMP (30 Ci/mmol) (Amersham) as a substrate. The assays were performed in 40 mM Tris–HCl (final pH 8.0), 10 mM $MgCl_2$ in the presence of 0.2 mM $CaCl_2$ and 15 nM human brain calmodulin (Calbiochem) at 37° for 10 min, and PDE activity was presented as picomoles cAMP hydrolyzed per minute per milligram protein. Total PDE activity represented the activity in the absence of inhibitors; cGMP-inhibited PDE (PDE3) or cAMP-specific PDE (PDE4) activity was defined as the inhibition of the activity by 10 μM cilostamide (a specific PDE3 inhibitor) or by 10 μM rolipram (a specific PDE4 inhibitor), respectively, as described [17]. PDE activity in the presence of 1 mM EGTA (a calcium chelator) and without Ca^{2+}/calmodulin was measured, and the difference from the total PDE activity was defined as Ca^{2+}/calmodulin-dependent PDE (PDE1) activity as described [18,19]. PDE activity in the presence of 10 μM cGMP was measured, and the inhibition by 10 μM EHNA was defined as cGMP-stimulated PDE (PDE2) activity as described [19]. The PDE1-, 2-, 3-, and 4-independent PDE activity was defined as PDE activity measured in the presence of 8-methoxymethyl-IBMX, EHNA, cilostamide, and rolipram (each 10 μM).

Table 1
Primers used for RT–PCR

Genes	Primer sequences	Product size (bp)	Ref.
PDE1B	5′-GCC TCA TCA GCC GCT TCA AGA TTC C-3′ 5′-GAA CTC CTC CAT TAG GGC CTT GG-3′	601	[19]
PDE4A	5′-AAC AGC CTG AAC AAC TNT AAC-3′ 5′-CAA TAA AAC CCA CCT GAG ACT-3′	907	[17]
PDE4B	5′-AGC TCA TGA CCC AGA TAA GTG-3′ 5′-ATA ACC ATC TTC CTG AGT GTC-3′	625	[17]
PDE4C	5′-TCG ACA ACC AGA GGA CTT AGG-3′ 5′-GGA TAG AAG CCC AGG AGA AAG-3′	289	[17]
PDE4D	5′-CCC TTG ACT GTT ATC ATG CAC ACC-3′ 5′-CCC TTG ACT GTT ATC ATG CAC ACC-3′	262	[18]
PDE7	5′-GGA CGT EGG AAT TAA GCA AGC-3′ 5′-TCC TCA CTG CTC GAC TGT TCT-3′	285	[23]
β-Actin	5′-GGG TCA GAA GGA TTC CTA TG-3′ 5′-GGT CTC AAA CAT GAT CTG GG-3′	268	[22]

2.5. Measurement of AC activity

The T cell lysate above was centrifuged at 23,600 *g* for 10 min at 4°. The pellet was used as a particulate fraction for AC assays as described [20,21]. The AC activity was measured at 37° for 10 min in 20 mM Tris–HCl (pH 7.4), 1 mM [α-^{32}P]adenosine triphosphate (30 Ci/mmol) (Amersham), 1 mM [^{3}H]cAMP, 1 mM IBMX, 5 mM $MgCl_2$, 0.2 mM EGTA, 20 mM creatine phosphate, and 100 U/mL of creatine phosphokinase. AC activity was presented as picomoles cAMP formed per minute per milligram protein.

2.6. RT–PCR

T cells were incubated under the conditions indicated, and total cellular RNA was extracted using a mRNA purification kit (Pharmacia). cDNA was made from RNA samples using Moloney mouse leukemia virus reverse transcriptase (Gibco-BRL) as described previously [22]. PCR was performed using primer sets (Table 1) in the thermal cycler programmed at 93° for 1 min, 60° for 1 min, and 72° for 2 min for 35 cycles. The PCR products were analyzed by electrophoresis on 2.5% agarose gels and stained with ethidium bromide. The intensity of the band for the RT–PCR products was determined by densitometry (Hoefer Scientific Instruments). Negative controls included samples in which cDNA synthesis was performed in the absence of reverse transcriptase and samples in which no cDNA was added during PCR.

2.7. Statistical analyses

One-way analysis of variance with Scheffe's multiple comparison test was used for the data in Fig. 5. One-way analysis of variance with Dunnett's multiple comparison test was used for the data in Table 2. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Inhibitory effects of AC and PDE inhibitors on IL-13 production

To clarify the involvement of AC and PDE isotypes in the PHA- or anti-CD3/CD28-induced IL-13 production, T cells were preincubated with AC or PDE isotype-specific inhibitors before stimulation, and their inhibitory effects on IL-13 production were examined. In PHA-stimulated T cells, the AC inhibitor MDL 12,330A blocked IL-13 production completely (IC_{50} = 0.1 μM) (Fig. 1A). The other

Table 2
Inhibitory effects of cAMP-modulating agents on PHA- or anti-CD3/CD28-induced IL-13 production

Agents	IL-13 production (pg/mL)	
	PHA	Anti-CD3/CD28
None	1202 ± 121	1452 ± 186
IBMX (0.5 mM)	43 ± 9* (98)	33 ± 6* (99)
Vinpocetine (50 μM)	483 ± 52* (61)	804 ± 73* (45)
EHNA (10 μM)	1172 ± 150 (3)	1430 ± 151 (2)
Milrinone (10 μM)	1162 ± 191 (3)	1420 ± 153 (2)
R0-20-1724 (10 μM)	732 ± 82* (40)	917 ± 98* (37)
SQ22536 (10 μM)	58 ± 9* (96)	81 ± 9* (95)
2′,5′-DDOA (10 μM)	68 ± 12* (95)	52 ± 7*(97)
Forskolin (100 μM)	21 ± 4* (99)	36 ± 5* (98)
8-bcAMP (1 mM)	34 ± 5* (98)	66 ± 9* (96)

T cells from five different donors (two men and three women) were preincubated with AC or PDE inhibitors at indicated concentrations for 30 min, and were stimulated with PHA (10 μg/mL) or anti-CD3/CD28 (each 0.1 μg/mL) as described in the legend of Fig. 1. After 48 hr, the culture supernatants were assayed for IL-13 by ELISA. Data are means ± SEM (N = 5). The values in parentheses are the percent inhibition calculated by the equation described in "Materials and methods." The IL-13 amount with medium alone was 13 ± 5 pg/mL (mean ± SEM, N = 5). Abbreviations: IBMX, 3-isobutyl-1-methylxanthine; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine; 2′,5′-dideoxyadenosine; and 8-bcAMP, 8-bromo-cAMP.

* Significantly different from controls with either stimulus alone ($P < 0.05$).

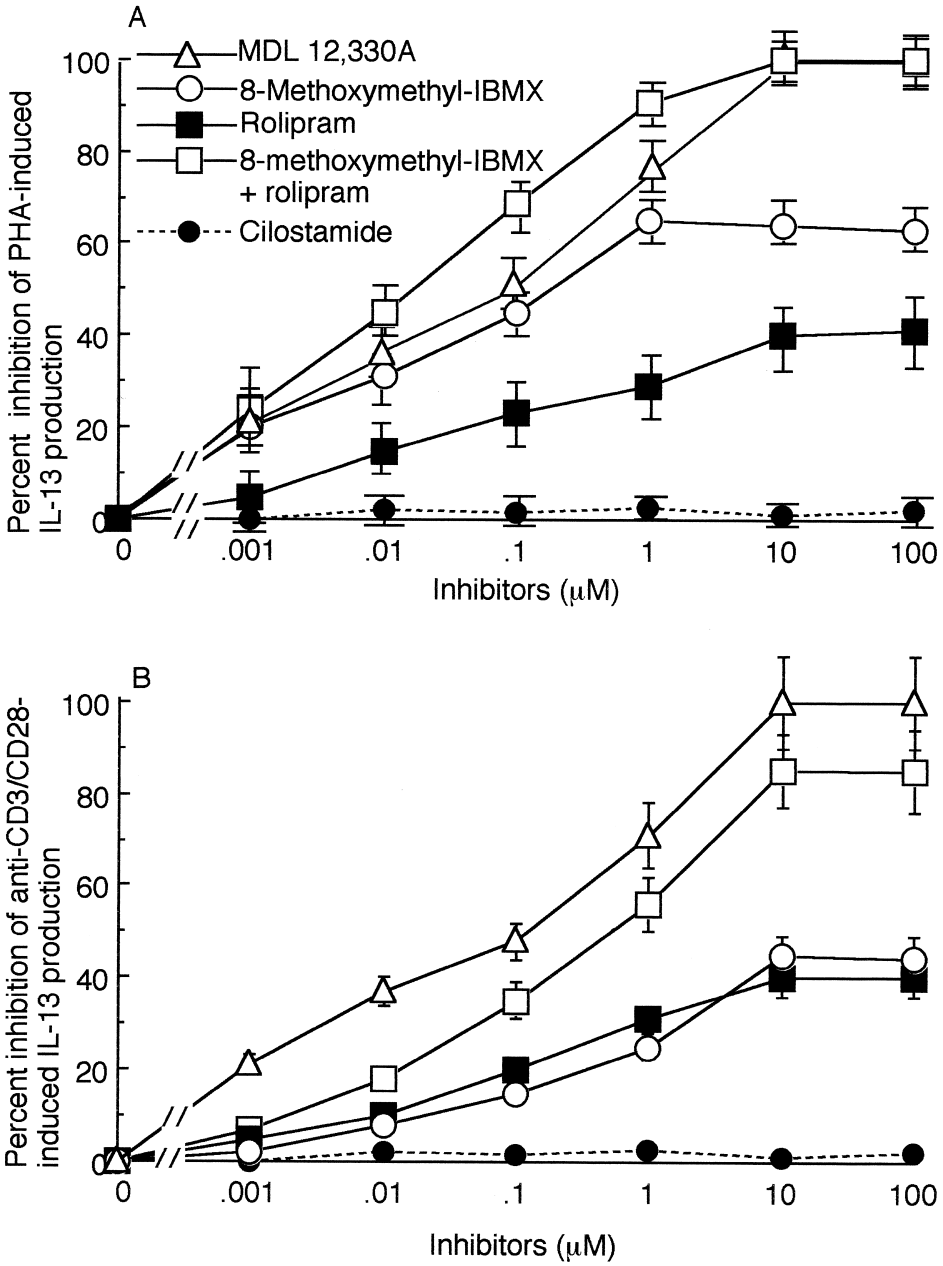

Fig. 1. Concentration-dependency for the inhibitory effects of AC or PDE inhibitors on IL-13 production. T cells from five different donors (two men and three women) were preincubated for 30 min with medium alone or with medium containing various AC or PDE inhibitors at the indicated concentrations in GAM-coated wells; then PHA (final concentration of 10 μg/mL) (A) or anti-CD3/CD28 (each 0.1 μg/mL) (B) was added. T cells were cultured with medium alone in parallel. After 48 hr, the amounts of IL-13 in the culture supernatants were measured by ELISA. Data are presented as percent inhibition calculated by the equation described in "Materials and methods." Values are means ± SEM (N = 5). The amount of IL-13 without inhibitors was as follows: 13 ± 5 pg/mL in medium alone, 1202 ± 121 pg/mL in PHA alone, and 1452 ± 186 pg/mL in anti-CD3/CD28 alone (mean ± SEM, N = 5).

AC inhibitors, SQ22536 and 2′,5′-dideoxyadenosine, also blocked the PHA-induced IL-13 production completely (Table 2). These results suggest the requirement of AC for PHA-induced IL-13 production. Among the PDE isotype-specific inhibitors, the PDE1 inhibitor 8-methoxymethyl-IBMX was the most inhibitory on IL-13 production (61% inhibition at 1 μM; Fig. 1A). The PDE4 inhibitor rolipram showed a smaller, but significantly inhibitory effect (38% inhibition at 10 μM). The usage of both 8-methoxymethyl-IBMX and rolipram gave additive inhibitory effects, and completely blocked PHA-induced IL-13 production when added at 10 μM each. Another PDE 1 inhibitor, vinpocetine, or the PDE4 inhibitor Ro-20–1724 also inhibited PHA-induced IL-13 production by a magnitude comparable to that of 8-methoxymethyl-IBMX or that of rolipram, respectively (Table 2). Neither the PDE3 inhibitor cilostamide (Fig. 1A) nor milrinone (Table 2) inhibited PHA-induced IL-13 production significantly. Likewise, the PDE2 inhibi-

tor EHNA did not have a significant inhibitory effect (Table 2). PHA-induced IL-13 production was blocked completely by the non-specific PDE inhibitor IBMX, the AC activator forskolin, or the cAMP analogue 8-bromo-cAMP (Table 2). These results indicate that a prolonged and excessive cAMP signal may suppress PHA-induced IL-13 production.

On the anti-CD3/CD28-induced IL-13 production (Fig. 1B, Table 2), the inhibitory effects of AC and PDE inhibitors were mostly comparable to those on PHA-induced IL-13 production; anti-CD3/CD28-induced IL-13 production was inhibited completely by AC inhibitors (IC_{50} of MDL 12,330A = 0.12 μM), and was partially blocked by PDE1 or PDE4 inhibitors, but not by PDE2 or PDE3 inhibitors. The inhibitory effect of 8-methoxymethyl-IBMX on anti-CD3/CD28-induced IL-13 production (45% inhibition at 10 μM) was slightly smaller than that on PHA-induced IL-13 production. The addition of both 8 methoxymethyl-IBMX and rolipram (each at 10 μM) inhibited the anti-CD3/CD28-induced IL-13 production by 85%; however, the combination did not inhibit IL-13 production completely even with further increased concentrations, indicating the possible contribution of another PDE isotype(s). These results suggest that both PDE1 and PDE4 may be required for PHA- or anti-CD3/CD28-induced IL-13 production, and the contribution of each PDE isotype to the IL-13 production seems to be independent. Anti-CD3/CD28-induced IL-13 production may possibly involve the other PDE isotype(s). We then tried to clarify at which time AC, PDE1, or PDE4 is required after the stimuli.

3.2. Time-dependence for the effects of AC, PDE, and PDE4 inhibitors on IL-13 production

MDL 12,330A, rolipram, or 8-methoxymethyl-IBMX was added to T cells at various time points before, simultaneously with, or after the stimuli, and the inhibitory effects on IL-13 production were compared. In PHA-stimulated T cells, the inhibitory effect of each agent was most potent when added before the stimulus, and decreased with delayed addition after the stimulus (Fig. 2A). The addition of MDL 12,330A 10 min after PHA did not inhibit IL-13 production at all, and further delayed addition was not inhibitory either (data not shown). This suggests that AC may be required only in the very early phase (<10 min) of the IL-13 induction. When rolipram was added 2 hr after PHA, its inhibitory effect was mostly lost, although a weak inhibitory effect (about 8% inhibition) was detected by the addition at 8 hr. This indicates that PDE4 may be involved mainly in the first 2 hr of IL-13 induction, although it may also be required in the later phase. The inhibitory effect of 8-methoxymethyl-IBMX was not reduced by addition 2 hr after PHA; however, the inhibitory effect gradually decreased with further delayed addition. This indicates that PDE1 may be required for the later phase (>2 hr) of IL-13 induction. In anti-CD3/CD28-stimulated T cells (Fig. 2B), the time-dependence for the inhibition by AC, PDE1, and PDE4 inhibitors was mostly equivalent to that in PHA stimulation. We then studied the kinetics of AC and PDE isozyme activities after the stimuli.

3.3. Kinetics for activities of AC and PDE isozymes

The AC activity of T cells rapidly increased, peaked at 5 min after PHA or anti-CD3/CD28 stimulation, and returned to the basal level at 1 hr (Fig. 3). Following the transient activation of AC, PDE4 activity increased and peaked at 30 min after either stimulus, and returned to the basal level at 2 hr (Fig. 4A). The transient activation of PDE4 was not blocked by actinomycin D or cycloheximide (Fig. 5A), indicating that the activation may not involve *de novo* protein synthesis. The activation of PDE4 was blocked by the cAMP-dependent protein kinase (protein kinase A) inhibitor H-89, indicating the involvement of protein kinase A. PDE1 activity was not detected in resting T cells, and was induced by PHA or anti-CD3/CD28 (Fig. 4B). PDE1 activity was detected at 3 hr after either stimulus, and gradually increased up to 24 hr. Although the PHA-induced PDE1 activity appeared to be slightly higher than that induced by anti-CD3/CD28, the difference was not significant. The PHA- or anti-CD3/CD28-induced PDE1 activity was suppressed by actinomycin D and cycloheximide (Fig. 5B), indicating the requirement of *de novo* protein synthesis. The induction of PDE1 was not blocked by H-89, indicating that protein kinase A may not be involved in the induction. PDE3 activity was not altered by either PHA or anti-CD3/CD28 (Fig. 4C). PDE2 activity was not detected in any of the control, PHA-, or anti-CD3/CD28-stimulated T cells over a 0- to 48-hr period (data not shown). At 24 hr, the sum of PDE1, 3, and 4 activities formed more than 85% of total PDE activity (Fig. 4D) in control, PHA-, or anti-CD3/CD28-stimulated T cells. However, a small proportion of PDE activity was insensitive to the inhibition by 8-methoxymethyl-IBMX, EHNA, cilostamide, and rolipram, i.e. independent of PDE1, 2, 3, and 4. In anti-CD3/CD28-stimulated T cells, the PDE1-, 2-, 3-, and 4-independent PDE activity (Fig. 4E) began to increase at 3 hr and peaked at 12 hr. Such an increase was not induced by PHA. At 12 hr, the proportion of PDE1-, 2-, 3-, and 4-independent PDE activity was 5, 2, or 15% of the total PDE activity in control, PHA- or anti-CD3/CD28-stimulated T cells, respectively. A recent paper proved the mRNA expression of cAMP-specific, rolipram-insensitive PDE (PDE7) in human peripheral T cells and indicated its enzymatic activity in these cells [23]. Since PDE7-specific inhibitor is not currently available, a 24-bp PDE7 antisense oligonucleotide (AS-O) (5′-CGGCAGCTGCTAACACACTTCCAT-3′) was synthesized [24] and was tested. As a control, a nonsense oligonucleotide (NS-O) containing a reversed sequence also was synthesized. In anti-CD3/CD28-stimulated T cells, the increase of PDE1-, 2-, 3-, and 4-independent PDE activity was blocked by PDE7 AS-O but not by PDE7 NS-O (Fig. 5C), suggesting that the activity may consist mostly of

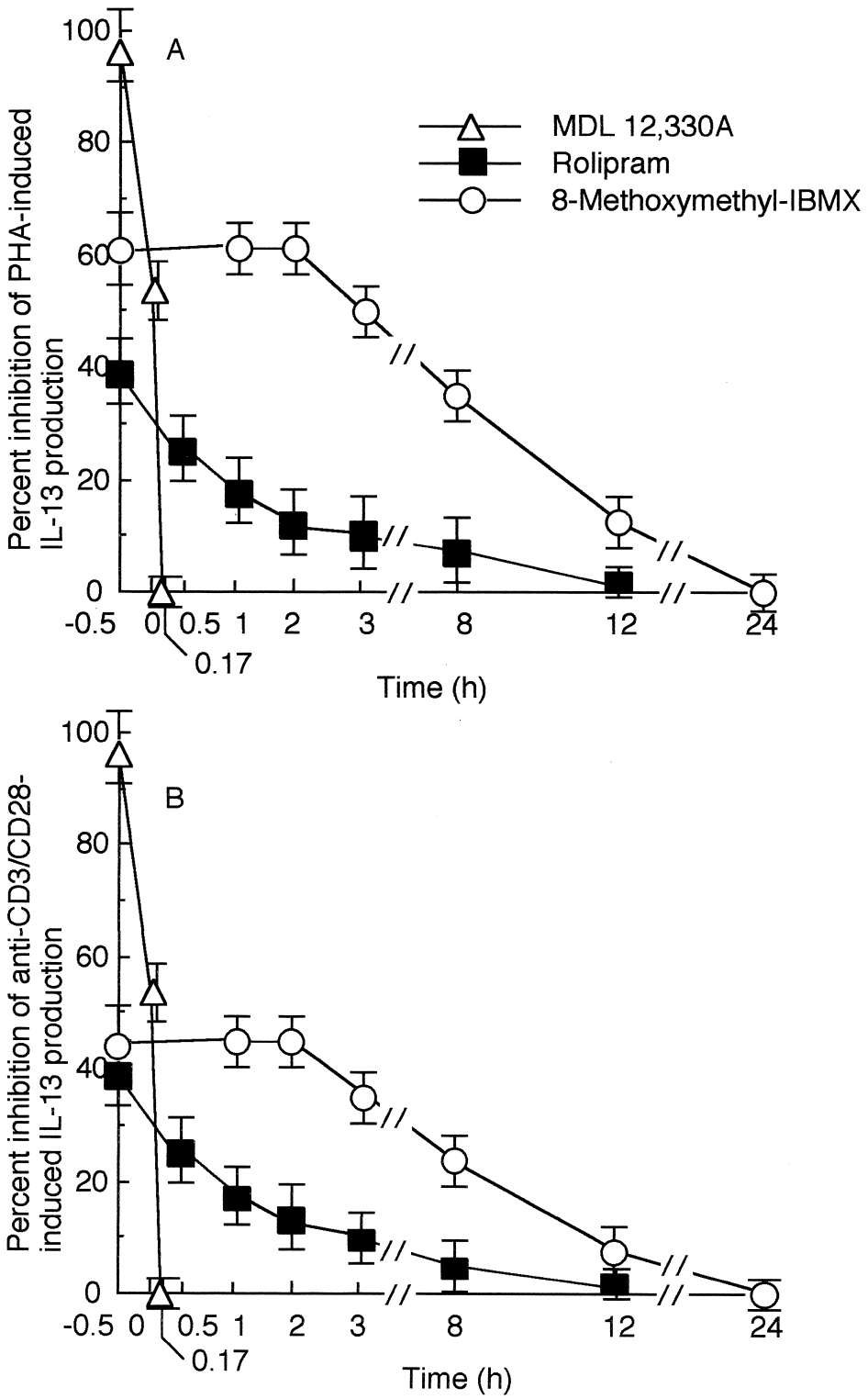

Fig. 2. Time-dependence for the inhibitory effects of AC or PDE inhibitors on IL-13 production. T cells from five different donors (two men and three women) were stimulated with PHA 10 μg/mL (A) or anti-CD3/CD28 (each 0.1 μg/mL) (B) in GAM-coated wells for 48 hr, and the amount of IL-13 in the culture supernatants was measured by ELISA. The time when the stimuli were added is defined as 0 hr. Rolipram or 8-methoxymethyl-IBMX (each 10 μM) was added 30 min before (-0.5 hr) or at the indicated time points after the stimuli. MDL 12,330A (10 μM) was added 30 min before (-0.5 hr), simultaneously (0 hr), or 10 min after the stimuli (0.17 hr). T cells were cultured with medium alone in parallel. Data are presented as percent inhibition calculated by the equation shown in "Materials and methods." The amount of IL-13 without inhibitors was as follows: 15 ± 4 pg/mL in medium alone, 1315 ± 148 pg/mL in PHA alone, and 1620 ± 216 pg/mL in anti-CD3/CD28 alone (mean $\pm$ SEM, $N = 5$).

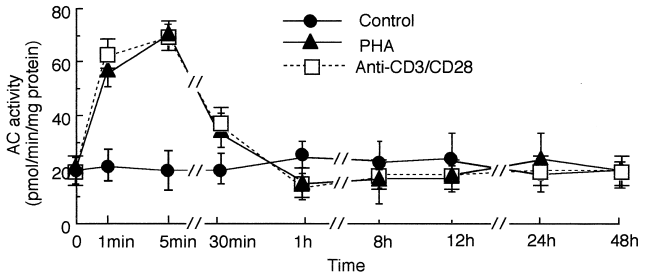

Fig. 3. Kinetics of AC activity. T cells from five different donors (two men and three women) were cultured with PHA (10 μg/mL) or anti-CD3/CD28 (each 0.1 μg/mL) in GAM-coated wells. T cells were cultured with medium alone in parallel. The AC activity was measured at the indicated time points after the stimuli. Values are means ± SEM (N = 5).

PDE7 [25]. The increase of PDE1-, 2-, 3-, and 4-independent PDE activity was blocked by actinomycin D and cycloheximide (Fig. 5C), indicating the requirement of *de novo* protein synthesis. The increase of PDE1-, 2-, 3-, and 4-independent PDE activity was not blocked by H-89, indicating that protein kinase A may not be involved in the increase.

We then analyzed PDE1, PDE4, and PDE7 mRNA levels in PHA- or anti-CD3/CD28-stimulated T cells. PDE1B mRNA expression, undetectable in resting T cells, was induced by PHA or anti-CD3/CD28 after 30 min of lag time, and the expression was detected over a 2- to 24-hr period (Fig. 6). The PDE1B mRNA expression was not blocked by H-89, suggesting that protein kinase A may not be involved in the expression. PDE4A mRNA expression was detected in resting T cells, and the mRNA level was not altered by either PHA or anti-CD3/CD28; the intensity ratio of PDE 4A/β-actin RT–PCR products in PHA- or anti-CD3/CD28-stimulated T cells was 94–104% of that in control T cells over a 0.5- to 24-hr period. The PDE4B and 4D mRNA levels were not increased by PHA or anti-CD3/CD28, either. PDE4C mRNA expression was not detected in any of control, PHA- or anti-CD3/CD28-stimulated T cells. Thus, PHA and anti-CD3/CD28 promoted PDE1 mRNA expression in T cells but not that of PDE4. PDE7 mRNA expression was detected in resting T cells, and the mRNA level was enhanced by anti-CD3/CD28 at 4 hr; the intensity ratio of PDE7/β-actin RT–PCR products in anti-CD3/CD28-stimulated T cells was 3-fold of that in control T cells. The enhancement of PDE7 mRNA expression was specifically inhibited by PDE7 AS-O, but not by PDE7 NS-O. When the RT–PCR reaction was performed without reverse transcriptase, no detectable bands were obtained on agarose gels with the primer pair for PDE4A (Fig. 6) or the other pairs (data not shown), indicating that the bands seen for amplified cDNA were not due to the contamination of genomic DNA. To clarify the roles of AC and PDE isozymes in the regulation of the cAMP level, we examined the kinetics of the level of cAMP after PHA or anti-CD3/CD28 stimulation.

3.4. Kinetics of the intracellular cAMP level in PHA- or anti-CD3/CD28-stimulated T cells

As shown in Fig. 7, the cAMP level of T cells increased 2.1- or 2.2-fold above the basal level at 5 min after PHA or

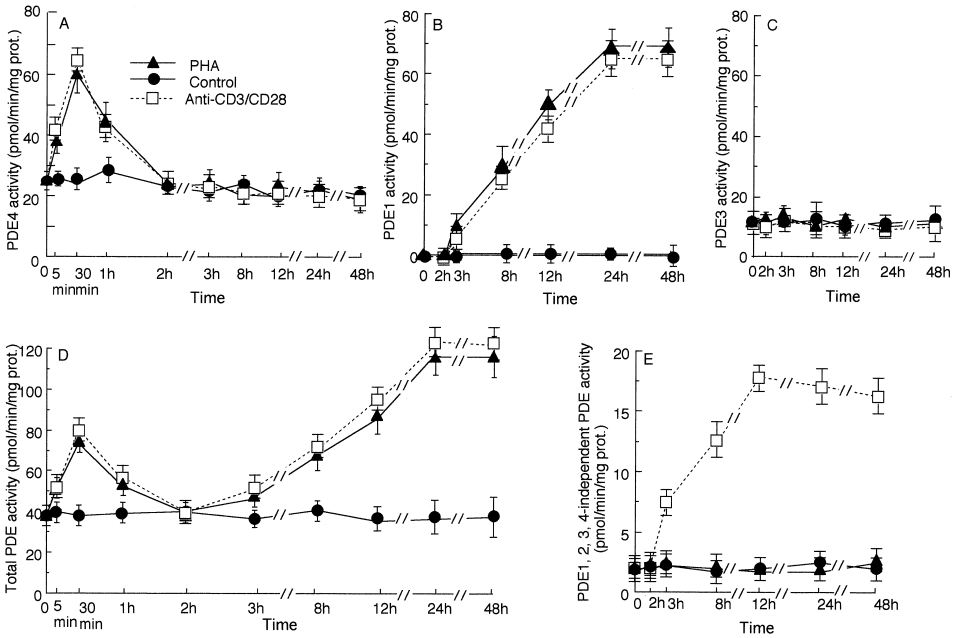

Fig. 4. Kinetics of total and isotype-specific PDE activities. T cells from five different donors (two men and three women) were cultured with PHA (10 μg/mL) or anti-CD3/CD28 (each 0.1 μg/mL) in GAM-coated wells. T cells were cultured with medium alone in parallel. PDE4 (A), PDE1 (B), PDE3 (C), total PDE activity (D), and PDE1-, 2-, 3-, and 4-independent PDE activity (E) were measured at the indicated time points as described in "Materials and methods." Values are means ± SEM (N = 5).

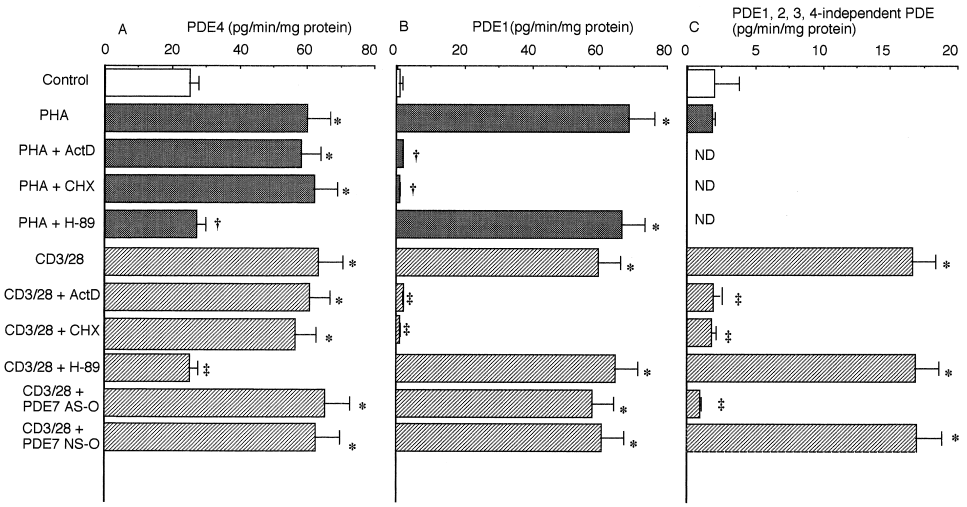

Fig. 5. Effects of various agents on PHA- or anti-CD3/CD28-induced increases of PDE4 (A), PDE1 (B), and PDE1-, 2-, 3-, and 4-independent PDE (C) activities. T cells from five different donors (two men and three women) were cultured for 30 min (A), 24 hr (B), or 12 hr (C) with PHA (10 μg/mL) or anti-CD3/CD28 (each 0.1 μg/mL) in GAM-coated wells in the presence or absence of actinomycin D (ActD, 10 μg/mL), cycloheximide (CHX, 10 μg/mL), H-89 (0.5 μM), PDE7 AS-O (20 μM), or PDE7 NS-O (20 μM). T cells were cultured with medium alone in parallel. Cells were lysed, and the activities of PDE subtypes were measured as described in "Materials and methods." Values are means ± SEM (N = 5). ND, not done. Key: (*) $P < 0.05$ vs the values of control T cells with medium alone, (†) $P < 0.05$ vs the values of T cells with PHA alone, and (‡) $P < 0.05$ vs the values of anti-CD3/CD28 alone.

anti-CD3/CD28, respectively, then returned to the basal level at 2 hr, and continued to decrease further up to 24 hr, when the cAMP amount in PHA- or anti-CD3/CD28-stimulated cells was 25 or 20% of the basal level, respectively. The early increase of cAMP was consistent with the early activation of AC (Fig. 3), and was inhibited concentration-dependently by MDL 12,330A (Fig. 8); the IC_{50} values of MDL 12,330A for the inhibition of cAMP increase were 0.09 or 0.15 μM in PHA- or anti-CD3/CD28-stimulated T cells, respectively, which was comparable to the IC_{50} values for the inhibition of IL-13 production (Fig. 1). These results indicate that the inhibition of IL-13 production by MDL 12,330A may be mediated by the inhibition of an early cAMP signal. Rolipram blocked the recovery from the elevated cAMP level for the first 2 hr after either stimulus, which is indicative of the transient activation of PDE4 in this phase (Fig. 4A). 8-Methoxymethyl-IBMX did not affect the kinetics of the cAMP level for the first 2 hr after either stimulus; however, it blocked the decrease of cAMP over a 2- to 48-hr period, which is consistent with the continuous activation of PDE1 in this phase (Fig. 4B). When both 8-methoxymethyl-IBMX and rolipram were added, the cAMP level remained elevated over a 48-hr period. These results suggest that PHA or anti-CD3/CD28 may initially (≤5 min) activate AC in T cells and increase cAMP. Thereafter, PDE4 and PDE1 may be successively activated and prevent prolonged cAMP accumulation; PDE4 may mainly reverse the early cAMP increase during the first 2 hr, while PDE1 may further reduce the cAMP level in the later phase. Both the time-dependent increase and decrease of cAMP may be required for IL-13 production.

4. Discussion

PHA or anti-CD3/CD28 transiently activated AC and generated cAMP signal in the early phase (≤5 min). PHA binds to the TCR/CD3 complex and CD2 on the T cell surface [26,27]. Although TCR/CD3 and CD2 do not directly couple to AC, PHA binding to these molecules triggers the activation of phospholipase C [28–30], and the phospholipase C-mediated signals activate AC [29]. The activated phospholipase C generates inositol 1,4,5-triphosphate and diacylglycerol; the former induces intracellular Ca^{2+} mobilization and the latter activates protein kinase C [29]. It has been reported that Ca^{2+} forms a complex with cytosolic calmodulin and that the Ca^{2+}/calmodulin complex binds to AC and activates this enzyme, while protein kinase C also activates AC by phosphorylation [31]. Thus, PHA may activate AC indirectly via the TCR/CD3- and CD2-mediated phospholipase C pathway [32]. Since the stimulation of CD28 also triggers the activation of phospholipase C [33], CD3 and CD28 cross-linking may lead to AC activation via phospholipase C in a manner mimicking PHA. PHA or anti-CD3/CD28 increased the T cell cAMP level about 2-fold above the background, which was a smaller effect than that by typical AC stimulators; 10 μM prostaglandin E_2 or 100 μM forskolin increased the T cell cAMP level 18- to 20-fold above the background [9,18]. However, PHA- or anti-CD3/CD28-induced cAMP signal was necessary for IL-13 production and, thus, may be at least one of the triggering signals for IL-13 induction. To date, the involvement of AC in IL-13 production has not been studied precisely although previous papers suggest that cAMP may be the initial signal for DNA synthesis in PHA-

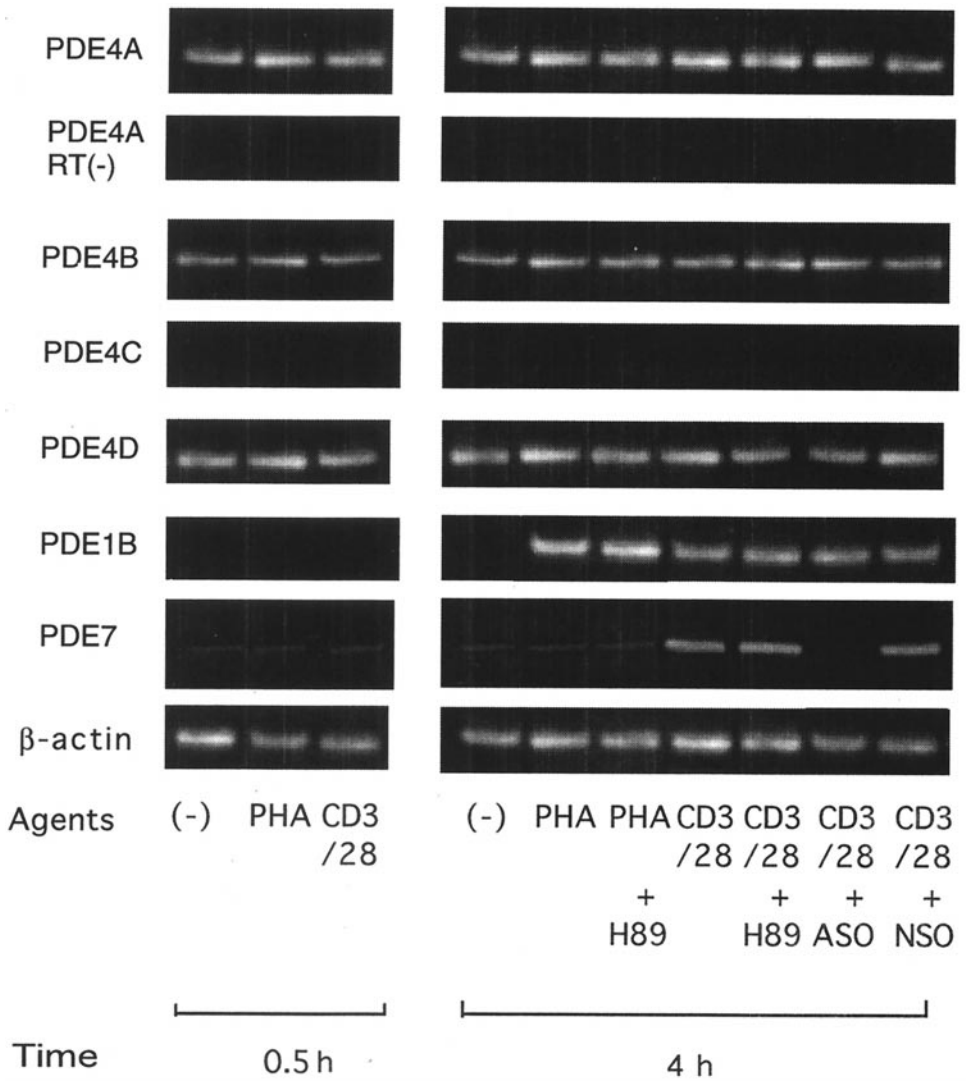

Fig. 6. RT–PCR analysis of PDE1, PDE4, and PDE7 mRNA expression. T cells from one healthy woman were cultured for 0.5 or 4 hr with PHA (10 μg/mL) or with anti-CD3/CD28 (each 0.1 μg/mL) in GAM-coated wells in the presence or absence of H-89 (0.5 μM), PDE7 AS-O (20 μM), or PDE7 NS-O (20 μM). RT–PCR was performed as described in "Materials and methods." Negative controls shown are the RT–PCR products for PDE4A performed without reverse transcriptase in cDNA synthesis (RT (-)). Data are representative of five separate experiments using T cells from five different donors (two men and three women).

stimulated T cells [34,35]. Since cAMP activates protein kinase A, this kinase may promote the early step of IL-13 production. One possible mechanism is that cAMP may induce the synthesis and/or activity of a certain transcription factor(s) crucial for IL-13 transcription. One candidate is AP-2 since an AP-2 binding site is present in the 5′-flanking region of IL-13 genes [36]. It has been reported that cAMP induces AP-2 transcription via protein kinase A in neuroectodermal cells [37,38]. It has also been suggested that cAMP may promote the interaction of AP-2 with another protein(s) to form more potent transcriptional complexes [39], or that cAMP may release an inhibitory protein(s), which may be constitutively associated with AP-2 and thus prevent the binding of AP-2 to DNA [40].

The PDE 1 and 4 activities in T cells increased following AC activation, and also were required for IL-13 production. PDE1 and 4 inhibitors prolonged cAMP accumulation, which appeared to suppress IL-13 production, thus suggesting that cAMP may inhibit the later events in IL-13 production. One possible mechanism is that cAMP may reduce the stability of IL-13 mRNA, which was also seen on IL-2 [41] or IL-4 mRNA in a previous study [10]. PDE1 and 4 may suppress cAMP accumulation and thus prevent the putative inhibitory effect of cAMP in the later phase.

The transient activation of PDE4 did not require *de novo* protein synthesis but involved protein kinase A. These findings indicate that protein kinase A, which is activated by the early cAMP signal, may activate PDE4 directly; it has been reported that protein kinase A phosphorylates PDE4D3 at serine 54, which induces rapid and reversible activation of PDE4D3 [42]. In the present study, the transiently activated PDE4 reversed the initial cAMP increase by hydrolysis,

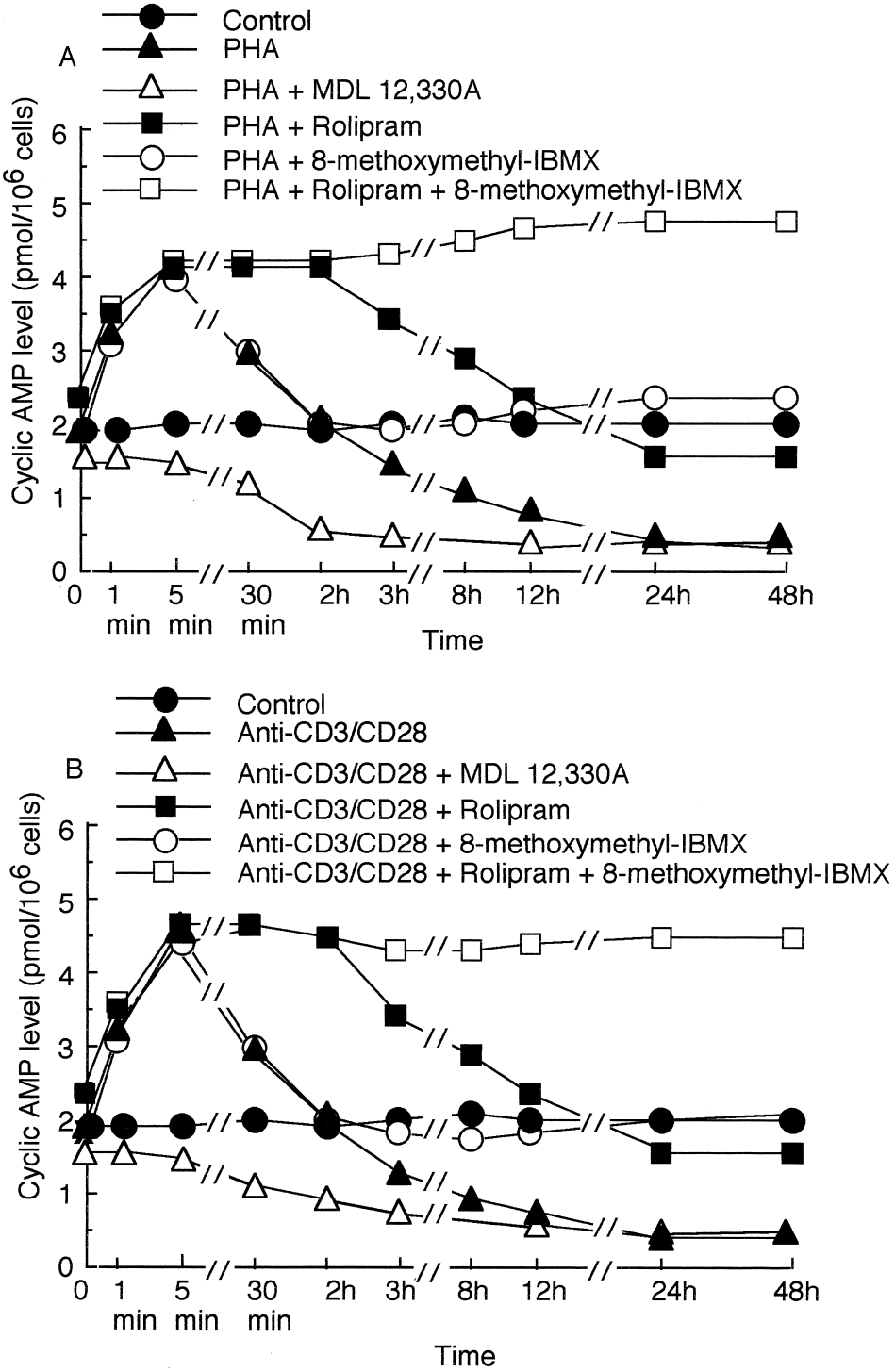

Fig. 7. Kinetics of the intracellular cAMP level. T cells from one healthy woman were preincubated for 30 min with medium alone or with medium containing MDL 12,330A (10 μM), or rolipram and/or 8-methoxymethyl-IBMX (each 10 μM) in GAM-coated wells; then PHA (final concentration of 10 μg/mL) (A) or anti-CD3/CD28 (each 0.1 μg/mL) (B) was added. T cells were cultured with medium alone in parallel. The intracellular cAMP level was analyzed at the indicated time points after the stimuli. The mean of triplicate cultures is shown; the SDs were $<$10% of the means. The data are representative of five separate experiments using T cells from five different donors (two men and three women).

indicating a negative feedback control of the level of cAMP. Recent studies have also reported that PDE4 transcription is promoted by a prolonged and excessive cAMP signal, 8-bromo-cAMP or forskolin-enhanced PDE4D transcription in T cells [18,43], indicating the presence of cAMP-inducible sites in the PDE4D promoter. In the present study, however, neither PHA nor anti-CD3/CD28 enhanced the level of PDE4D mRNA. This is possibly because the PHA-

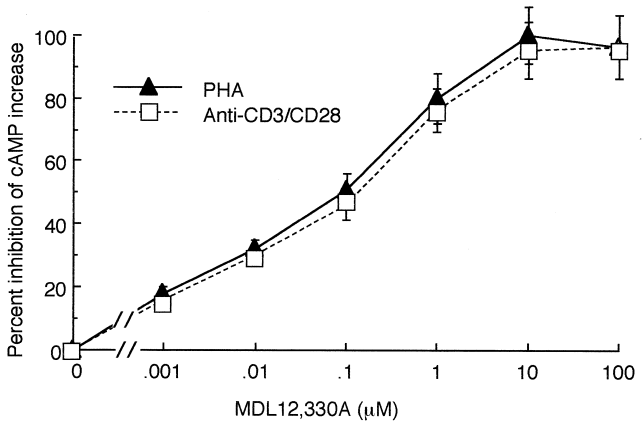

Fig. 8. Concentration-dependency for the inhibitory effects of MDL 12,330A on PHA- or anti-CD3/CD28-induced cAMP increases. T cells from five different donors (two men and three women) were preincubated for 30 min with medium alone or with medium containing MDL 12,330A at the indicated concentrations in GAM-coated wells; then PHA (final concentration of 10 μg/mL) or anti-CD3/CD28 (each 0.1 μg/mL) was added. T cells were cultured with medium alone in parallel. The intracellular cAMP level was analyzed at 5 min after the stimuli. Data are presented as percent inhibition calculated by the following equation: (cAMP amount with stimulus alone − cAMP amount with stimulus plus inhibitor) ÷ (cAMP amount with stimulus alone − cAMP amount with medium alone) × 100 (%). Values are means ± SEM (N = 5). The cAMP amount without MDL 12,330A was as follows: 2.1 ± 0.3 pmol/10^6 cells in medium alone, 4.3 ± 0.6 pmol/10^6 cells in PHA alone, and 4.6 ± 0.8 pmol/10^6 cells in anti-CD3/CD28 alone (mean ± SEM, N = 5).

or anti-CD3/CD28-induced cAMP signal was transient, and thus may be insufficient for the transcriptional induction.

The enzymatic activity and mRNA expression of PDE1, undetectable in resting T cells, were induced by PHA or anti-CD3/CD28. Protein kinase A was not involved in the PDE1B mRNA expression. Previous studies supported the hypothesis that PHA induces PDE1 mRNA expression and enzymatic activity [44,45], although PDE1 induction by anti-CD3/CD28 has not been reported. Our present results indicate that PDE1B transcription may possibly be induced by certain protein kinase A-independent signals mediated commonly by PHA and anti-CD3/CD28; one candidate is protein kinase C since both PHA and anti-CD3/CD28 activate protein kinase C via phospholipase C in T cells [28]. Spence *et al.* [19] also reported that the protein kinase C activator phorbol 12-myristate 13-acetate induces PDE1 mRNA expression in Chinese hamster ovary cells.

In this study, PDE7 mRNA expression in T cells was enhanced by anti-CD3/CD28 but not by PHA, which is consistent with results in recent studies [24,46]. It is thus indicated that PDE7 mRNA expression may be mediated via certain CD28-triggered signals that may not be induced by CD2 or CD3, such as acidic sphingomyelinase [47]. Anti-CD3/CD28 increased PDE1-, 2-, 3-, and 4-independent PDE activity while PHA did not. The increase of this activity was blocked by PDE7 AS-O, indicating the contribution of PDE7. However, we cannot verify that the PDE1-, 2-, 3-, and 4-independent PDE activity is authentically that of PDE7 due to the lack of a PDE7-selective inhibitor. Besides, we cannot currently examine if PDE7 AS-O can reliably abrogate PDE7 protein expression in anti-CD3/CD28-activated T cells by Western blot or similar experiments since PDE7-specific antibody is not available. In our preliminary studies, PDE7 AS-O slightly reduced IL-13 production in anti-CD3/CD28-activated T cells (approximately 15%), which was not statistically significant (data not shown). Thus, it still seems premature to conclude that PDE7 may regulate IL-13 production in anti-CD3/CD28-activated T cells. The possible role of PDE7 should be elucidated in future studies. The PDE1-, 2-, 3-, and 4-independent PDE activity may also consist of other isozymes different from PDE7, such as cAMP-specific IBMX-insensitive PDE (PDE8) [48], although the activity of these isozymes may be low. Since specific inhibitors for these isozymes are not currently available, we cannot examine their activities or those of PDE7 correctly. However, it is possible that these minor PDE isozymes may be related to IL-13 production in T cells. This possibility should be investigated further with developing selective inhibitors for the isozymes.

AC, PDE1, and PDE4 inhibitors suppressed IL-13 production. Since IL-13 is closely related to the development of atopic dermatitis, asthma, or allergic rhinitis [2,3], these agents can be used therapeutically for these allergic diseases. Patients with atopic dermatitis are particularly associated with increased PDE activity [49], which may be related to the increased IL-13 production in these patients. Therefore, it is expected that a PDE1 and/or PDE4 inhibitor may prevent the development of atopic dermatitis via the inhibition of IL-13 production. We are now studying the effects of these agents on IL-13 production by the T cells of these patients.

References

[1] Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes, and B cells, but not on T cells. Immunol Today 1994;15:19–26.

[2] Akdis M, Akdis CA, Weigl L, Disch R, Blaser K. Skin-homing, CLA^+ memory T cells are activated in atopic dermatitis, and regulate IgE by an IL-13-dominated cytokine pattern. IgG4 counter-regulation by CLA^- memory T cells. J Immunol 1997;159:4611–9.

[3] Huang SK, Xiao HQ, Kleine-Tebbe J, Paciotti G, Marsh DG, Lichtenstein LM, Liu MC. IL-13 expression at the sites of allergen challenge in patients with asthma. J Immunol 1995;155:2688–94.

[4] Li H, Sim TC, Alam R. IL-13 released by, and localized in human basophils. J Immunol 1996;156:4833–8.

[5] Jung T, Wijdenes J, Neumann C, de Vries JE, Yssel H. Interleukin-13 is produced by activated human $CD45RA^+$, and $CD45RO^+$ T cells: modulation by interleukin-4, and interleukin-12. Eur J Immunol 1996;26:571–7.

[6] Shichijo M, Shimizu Y, Hiramatsu K, Inagaki N, Takagi K, Nagai H. Cyclic AMP-elevating agents inhibit mite-antigen-induced IL-4, and IL-13 release from basophil-enriched leukocyte preparation. Int Arch Allergy Immunol 1997;114:348–53.

[7] Essayan DM, Kagey-Sobotka A, Lichtenstein LM, Huang SK. Regulation of interleukin-13 by type 4 cyclic nucleotide phosphodiester-

ase (PDE) inhibitors in allergen-specific human T lymphocyte clones. Biochem Pharmacol 1997;53:1055–60.
[8] Robicsek SA, Blanchard DK, Djeu JY, Krzanowski JJ, Szentivany A, Polson JB. Multiple high-affinity cAMP-phosphodiesterase in human T-lymphocytes. Biochem Pharmacol 1991;42:869–77.
[9] Wacholtz MC, Minakuchi R, Lipsky PE. Characterization of the 3′,5′-cyclic adenosine monophosphate-mediated regulation of IL2 production by T cells and Jurkat cells. Cell Immunol 1991;135:285–98.
[10] Borger P, Kauffman HF, Postma DS, Vellenga E. Interleukin-4 gene expression in activated human T lymphocytes is regulated by the cyclic adenosine monophosphate-dependent signaling pathway. Blood 1996;87:691–8.
[11] Epstein PM, Mills JS, Hersh EM, Strada SJ, Thompson WJ. Activation of cyclic nucleotide phosphodiesterase from isolated human peripheral blood lymphocytes by mitogenic agents. Cancer Res 1980; 40:379–86.
[12] Boyum A. Separation of leukocytes from peripheral blood and bone marrow. Scand J Clin Lab Invest 1968;21(Suppl 97):77–89.
[13] Gee AP, Lee C, Sleasman JW, Madden M, Ugelstad J, Barrett DJ. T lymphocyte depletion of human peripheral blood, and bone marrow using monoclonal antibodies, and magnetic microspheres. Bone Marrow Transplant 1987;2:155–63.
[14] Farrant J, Bryant AE, Lever AML, Edwards AJ, Knight SC, Webster ADB. Defective low-density cells of dendritic morphology from the blood of patients with common variable hypogammaglobulinaemia: low immunoglobulin production on stimulation of normal B cells. Clin Exp Immunol 1985;61:189–94.
[15] Verwilghen J, Vandenberghe P, Wallays G, de Boer M, Anthony N, Panayi GS, Ceuppens JL. Simultaneous ligation of CD5 and CD28 on resting T lymphocytes induces T cell activation in the absence of T cell receptor/CD3 occupancy. J Immunol 1993;150:835–46.
[16] Epstein PM, Moraski S, Hachisu R. Identification and characterization of a Ca^{2+}-calmodulin-sensitive cyclic nucleotide phosphodiesterase in a human lymphoblastoid cell line. Biochem J 1987;243: 533–9.
[17] Ekholm D, Mulloy JC, Gao G, Degerman E, Franchini G, Manganiello D. Cyclic nucleotide phosphodiesterases (PDE) 3 and 4 in normal, malignant, and HTLV-1 transformed human lymphocytes. Biochem Pharmacol 1999;58:935–50.
[18] Erdogan S, Houslay MD. Challenge of human Jurkat T-cells with the adenylate cyclase activator forskolin elicits major changes in cAMP phosphodiesterase (PDE) expression by up-regulating PDE3 and inducing PDE4D1 and PDE4D2 splice variants as well as down-regulating a novel PDE4A splice variant. Biochem J 1997;321:165–75.
[19] Spence S, Rena G, Sweeney G, Houslay MD. Induction of Ca^{2+}/calmodulin-stimulated cyclic AMP phosphodisterase (PDE1) activity in Chinese hamster ovary cells (CHO) by phorbol 12-myristate 13-acetate and by the selective overexpression of protein kinase C isoforms. Biochem J 1995;310:975–82.
[20] Choi EJ, Xia Z, Storm DR. Stimulation of the type III olfactory adenylyl cyclase by calcium and calmodulin. Biochemistry 1992;31: 6492–8.
[21] Salomon Y, Londons D, Rodbell M. A highly sensitive adenylate cyclase assay. Anal Biochem 1974;58:541–8.
[22] Llorente L, Richaud-Patin Y, Fior R, Alcocer-Varela J, Wijdenes J, Fourrier BM, Galanaud P, Emilie D. *In vivo* production of interleukin-10 by non-T cells in rheumatoid arthritis, Sjogren's syndrome, and systemic lupus erythematosus. Arthritis Rheum 1994;37:1647–55.
[23] Giembycz MA, Corrigan CJ, Seybold J, Newton R, Barnes PJ. Identification of cyclic AMP phosphodiesterases 3, 4 and 7 in human $CD4^+$ and $CD8^+$ T-lymphocytes: role in regulating proliferation and the biosynthesis of interleukin-2. Br J Pharmacol 1996;118:1945–58.
[24] Li L, Yee C, Beavo JA. CD3-, and CD28-dependent induction of PDE7 required for T cell activation. Science 1999;283:848–51.
[25] Michaeli T, Bloom TJ, Martins T, Loughney K, Ferguson K, Riggs M, Rodgers L, Beavo JA, Wigler M. Isolation and characterization of a previously undetected human cAMP phosphodiesterase by complementation of cAMP phosphodiesterase-deficient *Saccharomyces cerevisiae*. J Biol Chem 1993;268:12925–32.
[26] Leca G, Boumsell L, Fabbi M, Reinherz EL, Kanellopoulos JM. The sheep erythrocyte receptor and both α and β chains of the human T-lymphocyte antigen receptor bind the mitogen lectin (phytohaemagglutinin) from *Phaseolus vulgaris*. Scand J Immunol 1986;23:535–44.
[27] O'Flynn K, Krensky AM, Beverley PC, Burakoff SJ, Linch DC. Phytohaemagglutinin activation of T cells through the sheep red blood cell receptor. Nature 1985;313:686–7.
[28] van Tits LJH, Michel MC, Motulsky HJ, Maisel AS, Brodde O-E. Cyclic AMP counteracts mitogen-induced inositol phosphate generation, and increases in intracellular Ca^{2+} concentrations in human lymphocytes. Br J Pharmacol 1991;103:1288–94.
[29] Bihoreau C, Heurtier A, Enjalbert A, Corvaia N, Bensussan A, Degos L, Kordon C. Activation of the CD3/T cell receptor (TcR) complex or of protein kinase C potentiate adenylyl cyclase stimulation in a tumoral T cell line: involvement of two distinct intracellular pathways. Eur J Immunol 1991;21:2877–82.
[30] Kanner SB, Damle NK, Blake J, Aruffo A, Ledbetter JA. CD2/LFA-3 ligation induces phospholipase-Cγ1 tyrosine phosphorylation, and regulates CD3 signaling. J Immunol 1992;148:2023–9.
[31] Iyengar R. Molecular and functional diversity of mammalian G_s-stimulated adenylyl cyclases. FASEB J 1993;7:768–75.
[32] Carrera AC, Rincon M, De Landazuri MO, Lopez-Botet M. CD2 is involved in regulating cyclic AMP levels in T cells. Eur J Immunol 1988;18:961–4.
[33] Ward SG. CD28: a signaling perspective. Biochem J 1996;318:361–77.
[34] Krishnaraj R, Talwar GP. Role of cyclic AMP in mitogen induced transformation of human peripheral leukocytes. J Immunol 1973;111: 1010–7.
[35] Monahan TM, Marchard NW, Fritz RR, Abell CW. Cyclic adenosine 3′:5′-monophosphate levels and activities of related enzymes in normal and leukemic lymphocytes. Cancer Res 1975;35:2540–7.
[36] Smirnov DV, Smirnova MG, Korobko VG, Frolova EI. Tandem arrangement of human genes for interleukin-4 and interleukin-13: resemblance in their organization. Gene 1995;155:277–81.
[37] Philipp J, Mitchell PJ, Palipiero U, Fontana A. Cell type-specific regulation of expression of transcription factor AP-2 in neuroectodermal cells. Dev Biol 1994;165:602–14.
[38] Meaney MJ, Diorio J, Francis D, Weaver S, Yau J, Chapman K, Seckl JR. Postnatal handling increases the expression of cAMP-inducible transcription factors in the rat hippocampus: the effects of thyroid hormones and serotonin. J Neurosci 2000;20:3926–35.
[39] Garcia MG, Campillos M, Marina A, Valdivieso F, Vazquez J. Transcription factor AP-2 activity is modulated by protein kinase A-mediated phosphorylation. FEBS Lett 1999;444:27–31.
[40] Luscher B, Mitchell PJ, Williams T, Tjian R. Regulation of transcription factor AP-2 by the morphogen retinoic acid and by second messengers. Genes Dev 1989;3:1507–17.
[41] Anastassiou ED, Paliogianni F, Balow JP, Yamada H, Boumpas DT. Prostaglandin E_2, and other cyclic AMP-elevating agents modulate IL-2, and IL-2Rα gene expression at multiple levels. J Immunol 1992;148:2845–52.
[42] Sette C, Conti M. Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. J Biol Chem 1996;271: 16526–34.
[43] Seybold J, Newton R, Wright L, Finney PA, Suttorp N, Barnes PJ, Adcock IM, Giembycz MA. Induction of phosphodiesterases 3B, 4A4, 4D1, 4D2, and 4D3 in Jurkat T-cells and in human peripheral

blood T-lymphocytes by 8-bromo-cAMP and G_s-coupled receptor agonists. Potential role in β_2-adrenoreceptor desensitization. J Biol Chem 1998;273:20575–88.

[44] Jiang X, Li J, Paskind M, Epstein PM. Inhibition of calmodulin-dependent phosphodiesterase induces apoptosis in human leukemic cells. Proc Natl Acad Sci USA 1996;93:11236–41.

[45] Hurwitz RL, Hirsch KM, Clark DJ, Holocombe VN, Hurwitz MY. Induction of a calcium/calmodulin-dependent phosphodiesterase during phytohemagglutinin-stimulated lymphocyte mitogenesis. J Biol Chem 1990;265:8901–7.

[46] Bloom TJ, Beavo JA. Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants. Proc Natl Acad Sci USA 1996;93:14188–92.

[47] Boucher L-M, Wiegmann K, Futterer A, Pfeffer K, Machleidt T, Schutze S, Mak TW, Kronke M. CD28 signals through acidic sphingomyelinase. J Exp Med 1995;181:2059–68.

[48] Fisher DA, Smith JF, Pillar JS, St. Denis SH, Cheng JB. Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. Biochem Biophys Res Commun 1998;246:570–7.

[49] Hanifin JM, Chan SC. Monocyte phosphodiesterase abnormalities and dysregulation of lymphocyte function in atopic dermatitis. J Invest Dermatol 1995;105:84S–8S.