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Adenosine 3′,5′-Cyclic Monophosphate (cAMP)-Dependent Inhibition of IL-5 from Human T Lymphocytes Is Not Mediated by the cAMP-Dependent Protein Kinase A

Karl J. Staples,* Martin Bergmann,2* Katsuyuki Tomita,* Miles D. Houslay, † Ian McPhee, † Peter J. Barnes,* Mark A. Giembycz,* and Robert Newton3*

IL-5 is implicated in the pathogenesis of asthma and is predominantly released from T lymphocytes of the Th2 phenotype. In anti-CD3 plus anti-CD28-stimulated PBMC, albuterol, isoproterenol, rolipram, PGE2, forskolin, cholera toxin, and the cAMP analog, 8-bromoadenosine cAMP (8-Br-cAMP) all inhibited the release of IL-5 and lymphocyte proliferation. Although all of the above compounds share the ability to increase intracellular cAMP levels and activate protein kinase (PK) A, the PKA inhibitor H-89 failed to ablate the inhibition of IL-5 production mediated by 8-Br-cAMP, rolipram, forskolin, or PGE2. Similarly, H-89 had no effect on the cAMP-mediated inhibition of lymphocyte proliferation. Significantly, these observations occurred at a concentration of H-89 (3 μM) that inhibited both PKA activity and CREB phosphorylation in intact cells. Additional studies showed that the PKA inhibitors H-8, 8-(4-chlorophenylthio)adenosine-3′,5′-cyclic monophosphorothioate Rp isomer, and a myristilated PKA inhibitor peptide also failed to block the 8-Br-cAMP-mediated inhibition of IL-5 release from PBMC. Likewise, a role for PKG was considered unlikely because both activators and inhibitors of this enzyme had no effect on IL-5 release. Western blotting identified Rap1, a downstream target of the cAMP-binding proteins, exchange protein directly activated by cAMP/cAMP-guanine nucleotide exchange factors 1 and 2, in PBMC. However, Rap1 activation assays revealed that this pathway is also unlikely to be involved in the cAMP-mediated inhibition of IL-5. Taken together, these results indicate that cAMP-elevating agents inhibit IL-5 release from PBMC by a novel cAMP-dependent mechanism that does not involve the activation of PKA. The Journal of Immunology, 2001, 167: 2074–2080.

Asthma is a complex inflammatory disease characterized by eosinophil infiltration into the airways (1). IL-5 is thought to be a major cytokine involved in this eosinophilia (2) and is an important regulator of eosinophil differentiation, maturation, and survival (3, 4). Although IL-5 is produced by eosinophils, mast cells, and epithelial cells (5–7), it is T cells of the Th2 phenotype that are the major source (8, 9).

The most common therapy for acute asthmatic bronchospasm are β2-adrenoreceptor agonists, which elevate intracellular cAMP and promote smooth muscle relaxation (10). However, cAMP may also inhibit inflammatory cell proliferation and the release of proinflammatory cytokines (11–13). These properties have led to the proposal that cAMP-elevating drugs such as phosphodiesterase (PDE) inhibitors may also show anti-inflammatory potential in the treatment of inflammatory diseases such as asthma and chronic obstructive pulmonary disease (14, 15).

Classically, cAMP exerts its effects by activating the cAMP-dependent protein kinase (PK), PKA, which subsequently phosphorylates downstream effector proteins such as myosin L chain kinase and CREB (16–20). However, the role of PKA in the anti-inflammatory effects of cAMP is less well established. Furthermore, alternative mechanisms of cAMP action that involve cAMP binding to and activating small guanine nucleotide exchange factors (GEFs) have been described (21, 22). One such GEF, known as exchange protein directly activated by cAMP (Epac) can, when bound by cAMP, directly activate the small Ras-like GTPase Rap1 to elicit downstream responses (21).

We have recently shown inhibition of IL-5 release and mRNA by cAMP-elevating agents in PBMC (23). In the present study, we have used the T cell-specific stimuli anti-CD3 plus anti-CD28 to evoke a T cell-specific response, allowing us to examine the mechanism by which cAMP inhibits IL-5 release from T cells.

Materials and Methods

Reagents

Anti-CD3 (UCHT1) and anti-CD28 (CD28.2) Abs were purchased from BD PharMingen (Cambridge, U.K.). PGE2, 8-bromoadenosine cAMP (8-Br-cAMP), cholera toxin (CTX), and forskolin were obtained from Sigma (Poole, U.K.). Rolipram was obtained from Schering-Plough (Berlin, Germany). PD098059, SB203580, H-8, H-89, and a myristilated PKA inhibitor peptide; Rp-8-CPT-cAMP, 8-(4-chlorophenylthio)adenosine-3′,5′-cyclic monophosphorothioate Rp isomer; Sp-8-Br-PET-cGMPS, β phenyl-1,3′-etheno-8-bromoguanosine-3′,5′-cyclic monophosphorothioate Sp isomer; Rp-8-pCPT-cGMPS, 8-(4-chlorophenylthio)guanosine-3′,5′-cyclic monophosphorothioate Rp isomer; Brad1, 5-bromo-2′-deoxyuridine; MAPK, mitogen-activated protein kinase; M KK1, MAPK kinase 1; RBD, Ras binding domain; RafGDS, Raf guanine nucleotide stimulator protein; ERK, extracellular-regulated protein kinase.

Abbreviations used in this paper: PDE, phosphodiesterase; PK, protein kinase; GEF, guanine nucleotide exchange factor; Epac, exchange protein directly activated by cAMP; 8-Br-cAMP, 8-bromoadenosine cAMP; CTX, cholera toxin; PKI, PKA inhibitor peptide; Rp-8-CPT-cAMP, 8-(4-chlorophenylthio)adenosine-3′,5′-cyclic monophosphorothioate Rp isomer; Sp-8-Br-PET-cGMPS, β phenyl-1,3′-etheno-8-bromoguanosine-3′,5′-cyclic monophosphorothioate Sp isomer; Rp-8-pCPT-cGMPS, 8-(4-chlorophenylthio)guanosine-3′,5′-cyclic monophosphorothioate Rp isomer; Brad1, 5-bromo-2′-deoxyuridine; MAPK, mitogen-activated protein kinase; M KK1, MAPK kinase 1; RBD, Ras binding domain; RafGDS, Raf guanine nucleotide stimulator protein; ERK, extracellular-regulated protein kinase.
inhibitor peptide (PKI) were purchased from Calbiochem (Nottingham, U.K.). 8-(4-chlorophenylthio)adenosine-3',5'-cyclic monophosphorothioate Rp isomer (Rp-8-CPT-cAMP), β-phenyl-1, N²-etheno-8-bromoguanosine-3',5'-cyclic monophosphorothioate Sp isomer (Sp-8-BrPET-cGMPs), and 8-(4-chlorophenylthio)guanosine-3',5'-cyclic monophosphorothioate Rp isomer (Rp-8-pCPT-cGMPs) were obtained from Biolog Life Science Institute (Bremen, Germany).

Preparation and treatment of human PBMC
Mononuclear cells were prepared from peripheral blood of healthy human volunteers and cultured at a density of 3 x 10⁶ cells/ml for all experiments, as previously described (23). Cells were stimulated by the addition of anti-CD3 (UCHT1) and anti-CD28 (CD28.2) Abs, each at 500 ng/ml in solution, as previously described (23).

IL-5 ELISA
Supernatants from 3 x 10⁶ cells were harvested 24 h after treatment, and ELISA was performed as described by the manufacturer (BD Pharmingen). Human rIL-5 (R&D Systems, Minneapolis, MN) was used as a standard.

FACS analysis of proliferation
The proliferation assay was adapted from the method of Lu and Lane (24). PBMC (6 x 10⁶ cells/treatment) were cultured for 44 h in the presence or absence of treatments as indicated, and then 50 μM 5-bromo-2'-deoxyuridine (BrdU) (Sigma) was added for a further 4 h. After harvesting, cells were washed with ice-cold PBS and frozen in cold 70% (v/v) ethanol overnight at −20°C. Cells were washed with ice-cold PBS and incubated for 30 min at room temperature in 2 M HCl and 0.5% (v/v) Triton X-100. Cells were washed once with 0.1 M sodium tetraborate before washing with PBS, 0.5% (v/v) Tween 20, and 1% (w/v) BSA. Cells were then incubated for 30 min at room temperature with a mouse anti-BrdU Ab (Amersham Pharmacia Biotech, Piscataway, NJ). After washing, cells were incubated with FITC-labeled anti-mouse Ab (DAKO, Ely, U.K.) for 30 min at room temperature. After further washing, cells were resuspended in PBS, 200 μg/ml RNase A, and 50 μg/ml propidium iodide. Flow cytometric analysis (BD Biosciences, Oxford, U.K.) and gating of proliferating cells was performed as previously described (24).

PKA assays
PBMC (12 x 10⁶ cells/treatment) were harvested after 1 h, washed with HBSS, and assayed according to the method described by Giembycz and Diamond (25).

Rap1 activation assays
PBMC (24 x 10⁶ cells/treatment) were harvested after 30 min, washed with HBSS, and assayed as described previously (26, 27).

Western blotting
PBMC (24 x 10⁶ cells/treatment) were washed with HBSS and lysed on ice in 10 mM HEPES (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM DTT, and 0.1% (v/v) Nonidet P-40 supplemented with proteinase inhibitors. Total proteins (20 μg) were run on 10% SDS-PAGE and electroblotted onto nitrocellulose membranes (Amersham Pharmacia Biotech). Immunodetection of pan-CREB and phospho-CREB (New England Biolabs, Hitchin, Herts, U.K.) and Rap1 (Transduction Laboratories, Lexington, KY) was conducted according to the manufacturer’s instructions.

Statistical analysis
Analyses were performed using Kruskal-Wallis or Friedman ANOVA or Wilcoxon’s signed rank test as appropriate. Results were considered significant if p < 0.05 (***, p < 0.001; **, p < 0.01; and *, p < 0.05).

Results
Inhibition of IL-5 production by cAMP-elevating drugs
We have previously shown that costimulation of PBMC for 24 h with activating Abs directed to the CD3 and CD28 surface markers is a T cell-specific stimulus that produced measurable quantities of IL-5 (23). This IL-5 release was profoundly inhibited by the cAMP analog 8-Br-cAMP, the PDE4 inhibitor rolipram, the G₂₁ activator CTX, the adenylate cyclase activator forskolin, as well as by PGE₂ (Fig. 1A). In addition, the β₂-adrenoceptor agonists albuterol and isoproterenol also inhibited IL-5 release (Fig. 1B). Furthermore, dose dependence was shown for 8-Br-cAMP, rolipram, forskolin, and PGE₂ (Fig. 2A). These effects occurred at EC₅₀ values within the range expected for either stimulation of adenylate cyclase or inhibition of PDE4, as appropriate.

For many cAMP-dependent responses, particularly in regard to control of smooth muscle tone, simultaneous treatment with compounds that activate adenylate cyclase and inhibit PDE is predicted to result in a greatly enhanced level of cAMP to and lead to enhanced cAMP-dependent responses (28, 29). To test the relationship between activation of adenylate cyclase and PDE inhibition, cells were stimulated with anti-CD3 plus anti-CD28, and the effect of various concentrations of forskolin in the presence of rolipram was analyzed (Fig. 1C). Forskolin dose-dependently inhibited release of IL-5 (IC₅₀ = 0.95 μM) and, in the presence of rolipram (10 nM), which alone caused a ~30% inhibition of IL-5 release, there was no obvious change in the concentration-response relationship to forskolin (IC₅₀ = 0.64 μM), indicating that rolipram may be by distinct pathways and not via a common cAMP pool.

Effect of kinase inhibitors
Pretreatment of PBMC with 3 μM H-89 had no effect on the inhibition of anti-CD3 plus anti-CD28-induced IL-5 generation by

**FIGURE 1.** cAMP-elevating agents inhibit IL-5 production. A, Cells were incubated with anti-CD3 (500 ng/ml) plus anti-CD28 (500 ng/ml) and coincubated with rolipram (30 μM), 8-Br-cAMP (1 μM), CTX (2 μg/ml), PGE₂ (1 μM), and forskolin (1 μM). After 24 h, supernatants were harvested for IL-5 determination. Data are expressed as picograms per milliliter and are plotted as means ± SEM (rolipram, n = 9; 8-Br-cAMP, n = 7; CTX, n = 9; PGE₂, n = 9; and forskolin, n = 7). Significance was assessed using ANOVA (Kruskal-Wallis) with a Dunn’s post-test. B, Cells were stimulated as in A, described above, and treated with either albuterol or isoproterenol (1 μM) before IL-5 determination after 24 h. Data are expressed as a percentage of anti-CD3 plus anti-CD28-stimulated cells and are plotted as means ± SEM (albuterol, n = 11; and isoproterenol, n = 10). Significance was assessed using the Wilcoxon’s signed rank test. C, Cells were incubated with anti-CD3 and anti-CD28 as described above and coincubated with various concentrations of forskolin in the absence (□) or presence (◇) of rolipram (10 nM) as indicated. Supernatants were harvested at 24 h for IL-5 determination. Data are expressed as a percentage of anti-CD3 plus anti-CD28-stimulated cells and are plotted as means ± SEM (n = 3).
A number of reports have suggested that mitogen-activated protein kinases (MAPKs) may mediate some of the responses to cAMP. The selective MAPK kinase 1 (MKK1) inhibitor PD098059 and the p38 MAPK inhibitor SB203580, which show IC_{50} values of 2–7 and 0.6 μM, respectively, were therefore tested (35, 36). In each case, drug concentrations of 10 and 3 μM, respectively, were selected just above the IC_{50} level to minimize the possibility of nonspecific effects. Coincubation of PD098059 or SB203580 with anti-CD3 plus anti-CD28 and 8-Br-cAMP did not result in any reversal of the cAMP-mediated inhibition of IL-5 (Table I). However, incubation of PBMC with PD098059 inhibited anti-CD3 plus anti-CD28-induced IL-5 release by 60%, whereas SB203580 had no effect (Table I).

**Effect of cAMP and H-89 on T cell proliferation**

Using BrdU incorporation and FACS, CD3 plus CD28 costimulation resulted in a proliferative response in lymphocytes, which on account of the stimulus, must correspond to T cells (Fig. 3, A and B). A number of studies have shown that cAMP-elevating agents can inhibit T cell proliferation, and we have confirmed this in response to 8-Br-cAMP, forskolin, and rolipram (11, 37) (Fig. 3). However, the addition of H-89 (3 μM) did not prevent the antiproliferative effect of these drugs and, in fact, appeared to enhance the observed inhibition.

**H-89 inhibits PKA activity and CREB phosphorylation**

To verify that H-89 was having a functional effect on PKA activity, PBMC were coincubated with 8-Br-cAMP, rolipram, or forskolin in the presence or absence of H-89 (Fig. 4A). In each case, there was a significant increase in PKA activity that was abolished by H-89. Stimulation with anti-CD3 plus anti-CD28 alone had no effect on PKA activity (data not shown).

CREB is activated by PKA-dependent phosphorylation of Ser^{133} (19). Therefore, we used the phosphorylation status of this residue to further validate the effect of H-89. Following anti-CD3 plus anti-CD28 stimulation, no increase in CREB phosphorylation was observed (Fig. 4B). However, addition of 8-Br-cAMP markedly increased CREB phosphorylation, and this effect was abolished by H-89 (Fig. 4B).

**Effect of cAMP-elevating agents on Rap1 activation**

Recently, cAMP has been found to activate several GEFs that stimulate downstream GTPases and feed into MAPK signaling pathways (38). One such GEF is Epac, which can activate the Ral guanine nucleotide exchange factor (GEF) Rap1 (39). To investigate the possible role of the Epac/cAMP-GEF-Rap1-B-Raf pathway, Western blotting was performed for B-Raf. However, we found no evidence in PBMC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-5 Released (% of max stim)</th>
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<tbody>
<tr>
<td>Stim</td>
<td>100.0</td>
</tr>
<tr>
<td>Stim + 8-Br-cAMP</td>
<td>0.64 ± 0.64</td>
</tr>
<tr>
<td>Stim + 8-Br-cAMP + PD098059</td>
<td>0.13 ± 0.13</td>
</tr>
<tr>
<td>Stim + 8-Br-cAMP + SB203580</td>
<td>3.14 ± 1.96</td>
</tr>
<tr>
<td>Stim + PD098059</td>
<td>43.48 ± 6.13</td>
</tr>
<tr>
<td>Stim + SB203580</td>
<td>101.56 ± 9.31</td>
</tr>
</tbody>
</table>

* PBMCs were stimulated with anti-CD3 plus anti-CD28 (500 ng/ml each) (Stim) in the presence of either PD098059 (10 μM) or SB203580 (3 μM) alone or with PD098059 (10 μM) or SB203580 (3 μM) plus 8-Br-cAMP (1 mM). Supernatants were harvested after 24 h and IL-5 was measured by ELISA. Results are expressed as means ± SEM of three independent experiments.
lysates of the 68-kDa band that corresponds to B-Raf in positive control PC12 cells. Thus, B-Raf-dependent signaling seems unlikely. However, immunoreactive bands were observed at 40 and 50 kDa (Fig. 5A). These bands were not observed in PC12 lysates, and at present, it is not clear whether these represent breakdown products of B-Raf, closely related proteins, or simply nonspecific interactions.

To study the activation status of Rap1, the GTP-bound form of Rap1 was affinity purified and analyzed by Western analysis (26). This method exploits the ability of the active GTP-bound form of Rap1 to bind the Ras binding domain (RBD) of the Ral guanine nucleotide stimulator protein (RalGDS). The RalGDS-RBD is expressed as a GST fusion protein and purified from bacterial culture, which then allows the active Rap1 to be affinity purified (26, 27).

However, Rap1 activation in PBMC stimulated by anti-CD3 plus anti-CD28 appeared to be reduced. This was further reduced by 8-Br-cAMP, and the addition of H-89 seemed to reverse this effect. Although these data failed to reach statistical significance, it is clear that these results do not support a role for Rap1 in the inhibition of IL-5 by cAMP-elevating agents.

**Discussion**

cAMP was identified as a second messenger in the 1950s and was subsequently shown to exert many of its downstream effects via activation of PKA (40). However, this paradigm has now become so entrenched that many cAMP-driven effects are simply assumed to be PKA dependent.

G₁-coupled receptors such as β₂-adrenoceptors or the EP₂ and EP₄ prostanoid receptors (41, 42) directly activate adenylyl cyclase to produce a transient rise in intracellular cAMP (43). This results in activation of downstream cAMP-dependent effector molecules such as PKA. In this study, we have used separate G₁-coupled receptor-mediated stimuli, direct activation of G₁, direct activation of adenylyl cyclase, and inhibition of PDE4, a cAMP-specific PDE, as well as the cAMP analog 8-Br-cAMP to inhibit IL-5 release from CD3 plus CD28-stimulated PBMC. Taken together, the use of these mechanistically distinct means of elevating cAMP strongly supports a cAMP-dependent mechanism for the observed inhibition of IL-5. However, contrary to the dogma that has been accepted in regard to smooth muscle tone (28, 29), combined treatment with forskolin and rolipram failed to elicit any additional effect over a simple additive response (Fig. 1C). These data suggest that the response to each compound is mechanistically
distinct and, as such, requires careful examination. One explanation for this effect is that these responses are actually cAMP independent. However, we view this possibility as unlikely, given the nature of the various compounds, which include an active cAMP analog. A second, more likely, possibility is that these responses are indeed cAMP-dependent, but that there exists multiple cAMP-dependent effector mechanisms that are either spatially or temporally separated (44). Spatial separation or compartmentalization of cAMP pools is not a new hypothesis and has been invoked to explain numerous effects including the lack of a direct relationship between cAMP content and PKA activity in canine trachealis (45), the failure of rolipram to potentiate isoproterenol-induced relaxation of bronchial smooth muscle (46), and a lack of synergy between albuterol and rolipram in the repression of LPS-induced TNF-α from monocytes (47). Furthermore, in recent years, the specific activation and localization/colocalization of the multiple adenylate cyclases, PDE and PKA isoforms, and isoform splice variants have been clearly demonstrated (48–50). Thus, the activation and recruitment of the various components of the cAMP signaling pathway to precise intracellular sites via interaction with adapter or scaffold proteins may produce levels of specificity that were previously unsuspected and could easily account for the observations in this study.

A second explanation for the lack of synergy between forskolin and rolipram may lie in the temporal separation between the cause and effect. Elevation of cAMP within T cells occurs in the order of minutes (51), whereas IL-5 release was measured after 24 h. Thus, the relationship between cause and effect is unlikely to be direct and could involve multiple downstream effectors and possible de novo gene expression. Thus, confounding factors, including the various forms of desensitization and other negative feedback processes, may obscure any synergy. In addition, there is evidence that cAMP may act at multiple levels (transcription, posttranscription, or translation) to repress gene expression. For example, the ability of albuterol to inhibit eotaxin release from smooth muscle was lost if added later than 2 h following cell stimulation, suggesting a window of effectiveness that corresponds to early gene expression events such as transcription (52). Similarly, cAMP-elevating agents are known to repress NF-κB-dependent transcription by a variety of mechanisms (53). This contrasts with the ability of β2-agonists to repress their own mRNA expression by posttranscriptional destabilization and the finding that, in adipocytes, cAMP-elevating agents down-regulated pathways that are involved in translational control (54, 55). Furthermore, one mechanism of growth inhibition by forskolin in lymphoid cells involved translational down-regulation of cyclin D3 (56). Thus, temporal separation may also be sufficient to account for our observations.

To explore the role of PKA in these responses, the effect of H-89, a selective inhibitor of PKA that acts by competitive inhibition of the ATP binding site, was assessed (30). However, no obvious effect in regard to IL-5 inhibition by 8-Br-cAMP, rolipram, forskolin, or PGE₂ was observed. Because this finding was shared with other structurally and mechanistically distinct inhibitors of PKA, these data suggest that PKA may not play a major role in this process. Furthermore, inhibition by H-89 of the cAMP-dependent increase in both PKA activity and phosphorylation of the PKA substrate, Ser323 of CREB, confirms this hypothesis by showing that H-89 gained entry to the cells and was functionally active.

In addition to effects on cytokine release, another important property of cAMP-elevating agents is their ability to inhibit T cell proliferation (11, 37). In this respect, we have shown that the inhibition of T cell proliferation by various cAMP-elevating agents was again independent of PKA. This finding is in agreement with a recent study, in which evidence for a PKA-independent mechanism in the immunomodulatory effects of cAMP was reported, and suggests that non-PKA-dependent effects play a major role in the cAMP-dependent effector functions in T cells (57).

Collectively, the results of the present study indicate that other cAMP-driven, but PKA-independent, signaling mechanisms account for the suppression of IL-5 production from PBMC. One possible candidate for these effects is the PKG signaling pathway. PKG, which is expressed in T cells, can be directly activated by cAMP, and activation of this enzyme has been shown to inhibit IL-2 generation (33, 34). In addition, PKG may also be indirectly activated via the cAMP-dependent induction of inducible NO synthase (58–60). This enzyme produces NO, which activates soluble guanylate cyclase, which in turn increases intracellular cGMP levels and activates PKG (61). However, as the active cGMP analog, Sp-8-Br-PET-cGMPS, and the selective PKG inhibitor, Rp-8-pCPT-cGMPS, both of which have biological activity in T cells (34), had no effect on IL-5 release or on cAMP-mediated inhibition of IL-5 release, respectively, our data suggest that this effect may not be PKG mediated.

Various MAPK pathways have been implicated as downstream effectors of cAMP (62). However, use of the MKK1 inhibitor, PD98059, and p38 MAPK inhibitor, SB203580, indicated that these particular pathways are not involved in the cAMP-mediated inhibition of IL-5. This is consistent with the downstream kinase, extracellular-regulated protein kinase (ERK), being involved in TCR-stimulated release of IL-5 (63, 64). Furthermore, and in agreement with previous studies (65), we have found that 8-Br-cAMP failed to inhibit the anti-CD3 plus anti-CD28-induced rise in ERK phosphorylation (data not shown). These observations, coupled with the fact that PD098059 inhibited IL-5 release by
~60%, support a role for MKK1 in the CD3 plus CD28-dependent release of IL-5 rather than in inhibition of IL-5 release.

Other putative targets of cAMP are the cAMP-activated GEFs Epac1 and Epac2 (22, 66). In PC12 cells, these GEFs activate Rap1 to signal through the Rap pathway (21, 66). Previous studies have suggested that B-Raf, which can activate ERK, is the predominant downstream effector of Rap1 (38). However, because we failed to detect B-Raf in PBMC, together with the involvement of MKK-ERK activation in the release of IL-5, the above scheme is unlikely in T cells (63, 64). Furthermore, because 8-Br-cAMP appeared to inhibit activation of Rap1, and this effect was reversed by H-89 (Fig. 5B), we tentatively suggest that Rap1 activation may be negatively regulated by a PKA-dependent mechanism. These data are in contrast to the pathway described in platelets, in which a similar assay was used to show cAMP activation of Rap1 (67). Certainly, we did not observe any activation of Rap1 and can therefore exclude a role for B-Raf and Rap1 in cAMP-mediated inhibition of IL-5 release. However, this observation does not rule out roles for the Epacs/cAMP-GEFs, which may act via different downstream effectors in these cells (66).

In summary, this study excludes a role for PKA and PKG in the cAMP-dependent inhibition of IL-5 release and proliferation of T cells in a mixed PBMC population. In addition, evidence is also presented that the downstream effectors of Epac/cAMP-GEF Rap1, and B-Raf are unlikely to be involved in the signaling downstream of cAMP in this system. Collectively, these data are highly important because, in addition to excluding a role for the above pathways, and in particular PKA, the data suggest that there is at least one uncharacterized cAMP-dependent pathway that mediates key cAMP-dependent responses in human T lymphocytes.

References

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