Cyclic AMP Modulates the Functional Plasticity of Immature Dendritic Cells by Inhibiting Src-like Kinases through Protein Kinase A-mediated Signaling*

Immature dendritic cells (iDCs) can be instructed to polarize the immune response toward a noninflammatory pathway by mediators that increase the intracellular concentration of cAMP. This phenomenon is associated with the ability of the cyclic nucleoside to inhibit the release of pro-inflammatory cytokines without affecting the differentiation process of the dendritic cells (DCs). Here we investigated the ability of cAMP to modulate the endotoxin signaling by exposing DCs to exogenous 8-bromo-cyclic AMP in the presence or absence of H89, a selective inhibitor of the protein kinase A, one of the major molecular targets of the cyclic nucleoside. cAMP affects the early lipopolysaccharide-induced signaling cascade dissociating the activation of NF-κB, p38, and ERK pathways from the stimulation of c-Src and Lyn kinases. This phenomenon was prevented by H89. The pharmacological block of Src-like tyrosine kinases induces comparable results confirming the involvement of this family of enzymes in the mechanism controlling the release of cytokines in human monocyte-derived iDCs. We propose that the cAMP-protein kinase A-dependent pathway regulates the functional plasticity of iDCs by gating the Toll-like receptor signaling at the level of Src-like kinases.

Dendritic cells (DCs) are antigen-presenting cells distributed in the blood, peripheral tissues, and lymphoid organs with a unique ability to activate and polarize naive T-cells (1–2). In peripheral tissues, DCs display an immature phenotype characterized by a high rate of endocytosis and low antigen-presenting capability. Upon encountering microbial products or inflammatory cytokines, immature DCs (iDCs) undergo a complex maturation process. They reduce the antigen uptake capability and up-regulate the cell surface expression of a variety of proteins including major histocompatibility complex (MHC) class I and class II and adhesion and costimulatory molecules. Maturation of DCs is also associated with changes in the cell surface expression of several chemokine receptors (3). This phenomenon is crucial for their migration from inflamed tissues to regional lymph nodes. The continuous traffic of T-lymphocytes through the paracortical areas of lymph nodes makes this a prime site for antigen-loaded DCs to encounter the low frequency, antigen-specific naive T-cells and to initiate the immune response. The antigen-presenting capacity of DCs critically depends on their state of maturation that determines the extent of T-cell activation as well as the type of T-cell response.

A number of facts have shown that DCs can be not only immunogenic but also tolerogenic, both intrathymically (4–5) and in the periphery (6). DCs display a functional plasticity (7) and can be instructed to polarize T-cells by mediators that are present in the peripheral environment. These factors affect the differentiation process of the iDCs, the exit of the mature DCs (mDCs) from the inflamed tissues (8), and the production of cytokines by mDCs after they have migrated to the lymph nodes (9). Intracellular cAMP is an important physiological mediator of the inflammation process, and it also plays a central role in the regulation of the immune response. Physiological and pharmacological agents that increase cAMP levels, such as prostaglandin E2 (PGE2) (10–13), histamine (14, 15), β2-adrenergic receptor agonists (16), extracellular ATP (17–19), and bacterial toxins (20), are able to interfere with the differentiation process of DCs via the G protein-adenylyl cyclase pathway. These agents display different effects on the maturation process of iDCs and share the capability of blocking the release of pro-inflammatory cytokines, including IL-12 and TNF-α, as well as stimulating IL-10 secretion by cAMP-dependent or -independent mechanisms (10–20). In the present study, we investigate the ability of cAMP to modulate the early signaling initiated by bacterial lipopolysaccharide (LPS). Because protein kinase A (PKA) is one of the molecular targets of the cyclic nucleoside, we also tested the ability of H89, a selective PKA inhibitor, to prevent the cAMP-mediated effects. We found that cAMP was able to uncouple the NF-κB, p38, and ERK pathways from the activation of Src family kinases. The modified signaling cascade generated in the presence of high intracellular levels of cAMP was able to support the matura-
tion process and the synthesis of IL-10, but it was not sufficient to induce the release of pro-inflammatory cytokines. Significantly, these effects were prevented by preincubating DCs with H89. These findings shed new light on the role of the cAMP/PKA pathway in the early biochemical events regulating the process of dendritic cell maturation.

**EXPERIMENTAL PROCEDURES**

**Media and Reagents**—The medium used throughout was RPMI 1640 (Invitrogen) supplemented with 2 mM l-glutamine, 50 ng/ml streptomycin, 50 units/ml penicillin, and 10% heat-inactivated fetal calf serum (HyClone Laboratories, Logan, UT) or 2% human serum (Sigma). Granulocyte–macrophage colony stimulating factor (GM-CSF) and IL-4 was obtained from PeproTech (Rocky Hill, NJ). 8-Br-cAMP and FGE, were purchased from Sigma and used at 200 and 1 μM, respectively. H89 and PP2 were purchased from Calbiochem and used at 10 μM.

In Vitro Generation and Culture of Human DCs—DCs were generated from peripheral blood mononuclear cells, as described (21), with some modification. Briefly, peripheral blood mononuclear cells were obtained from 30 ml of leukocyte-enriched buffy coat from healthy donors by centrifugation with F Lymphoprep gradient (Axis-Shield PoC AS, Oslo, Norway), and the light density fraction was recovered. Monocytes were isolated by positive selection using anti-CD14 conjugated magnetic microbeads (Miltenyi Biotec, Bologna, Italy). CD14+ cells were cultured at a concentration of 0.5–1 × 10⁶ cells/ml in RPMI 1640 supplemented with 10% fetal calf serum or 2% human serum (Sigma), 10% FCS, and IL-4. After washing, cells were incubated with avidin-FITC (BD Biosciences) and incubated with biotinylated anti-rabbit Ig antibodies (BD Biosciences) for 30 min at 4 °C. After washing, cells were incubated with an anti-CCR7 antibody (BD Biosciences) and type-matched control antibody from BD Biosciences. For indirect staining, cells were stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences. The cells were also stained with the corresponding FITC- or phycoerythrin-conjugated isotype-matched control antibody from BD Biosciences.
Here we investigated the effects of high intracellular levels of cAMP on the LPS-generated signaling. As expected, the incubation of iDCs for 24–48 h with 100 ng/ml endotoxin was associated with up-regulation of CD80, CD86, CD54, HLA-DR, and the de novo expression of CD83 (Fig. 1A). Addition of 8-Br-cAMP (200 μM) to culture medium did not affect CD86, CD83 and HLA-DR up-regulation but inhibited the increase of CD54 expression induced by LPS (Fig. 1A).

Because the switch in chemokine receptor expression on DCs is crucial to regulate their traffic from the inflamed tissues to the lymph nodes (3), we investigated the effect of 8-Br-cAMP on this event. Immature DCs expressed low levels of CCR5 and undetectable levels of CCR7 and CXCR4 as measured by flow cytometry (Fig. 1B). Stimulation by LPS resulted in a marked up-regulation of CCR7 accompanied by a decrease in the levels of CCR5 expression. 8-Br-cAMP did not affect the LPS-induced modulation of CCR5 and CCR7 but induced, even when added alone, a marked up-regulation of CXCR4 (Fig. 1B, and data not shown). To investigate whether these changes were generated by variations at the transcriptional level, we evaluated CCR5, CXCR4, and CCR7 mRNA levels by reverse transcriptase-PCR (Fig. 1C). Stimulation by LPS induced accumulation of CCR7 and CXCR4 mRNAs and, conversely, decreased CCR5 mRNA levels (Fig. 1C). 8-Br-cAMP did not block the LPS-induced modulation of CCR5 and CCR7 and increased the LPS-induced accumulation of CXCR4 mRNA (Fig. 1C). Finally, we verified the capability of DCs to migrate toward a chemokine gradient. As expected, immature DCs responded to RANTES but did not migrate toward SDF-1 and SLC (Fig. 1D). Upon stimulation by LPS, DCs acquired the ability to migrate toward SDF-1 and SLC but failed to respond to RANTES. DCs exposed to LPS in the presence of 8-Br-cAMP showed a higher capability to migrate toward RANTES, SDF-1, and SLC than immature or LPS-stimulated DCs.

Therefore, the high intracellular level of cAMP, obtained by culturing DCs in the presence of 8-Br-cAMP, does not prevent most of the events associated with their typical terminal differentiation process, including up-regulation of costimulatory and HLA-DR molecules, de novo expression of CD83, and switch in chemokine receptors.

**cAMP Blocks the Release of the Pro-inflammatory Cytokines and Impairs the Ability of DCs to Polarize a T-cell Response toward the Th1 Profile**—In response to bacterial endotoxin, immature DCs are able to release a complex mixture of immunomodulatory factors including cytokines, chemokines, and arachidonic acid derivatives (1–3). The relative amount of pro-inflammatory and regulatory mediators released accounts for the ability of mature DCs to promote inflammation or, conversely, to instruct the immune response toward a tolerogenic pathway. Thus, cytokines such as TNF-α and IL-12 favor local inflammation and Th1 response, whereas IL-10 attenuates the inflammatory response and blocks DCs migration to the draining lymph nodes (8). In our experimental conditions, stimulation of iDCs by LPS induced the release of TNF-α, IL-12, and IL-10 (Fig. 2A). The presence of 8-Br-cAMP resulted in a dose-dependent inhibition of TNF-α and IL-12 secretion but did not impair IL-10 production (Fig. 2A). Of note, at low dosage (2–20 μM) 8-Br-cAMP increased the LPS-induced IL-10 secretion (Fig. 2A).

Because IL-12 is a key cytokine for the induction of the pro-inflammatory type of immune response, DCs induced to mature by LPS in the presence of 8-Br-cAMP should be able to induce naïve T-cell proliferation but unable to drive efficient Th1 differentiation. To test this hypothesis, we stimulated CD4+ naïve T-cells with increasing doses of TSST-1 in the presence of fresh autologous monocytes or LPS-treated DCs exposed or not to 8-Br-cAMP. As expected, LPS-pulsed DC, but not monocytes, were able to stimulate allogeneic naïve T-cells to proliferate, whether or not they were pretreated with 8-Br-cAMP (data not shown). On the contrary, the presence of 8-Br-cAMP modulated the capability of both autologous and allogenic DCs to polarize the differentiation program of naïve T-cells toward the Th1 phenotype by decreasing the ratio of IFN-γ/IL-4-producing T-cells (Fig. 2B).

**cAMP Does Not Impair the Activation of NF-κB, p38, and ERK-1/2 Pathways Induced by LPS**—NF-κB is a ubiquitously
expressed family of transcription factors that control the expression of a number of genes involved in inflammation, immune response, and protection from apoptosis (22). In most cell types NF-κB is retained in the cytoplasm bound to the inhibitory subunit IκB. Upon stimulation of cells with different agents, such as inflammatory cytokines, IκB is phosphorylated on specific serine residues, polyubiquitinated, and degraded through a proteosome-dependent pathway. The released NF-κB dimer translocates rapidly to the nucleus where it activates the transcription of target genes (20). To investigate if cAMP was able to interfere with the activation of NF-κB mediated by LPS, we performed an EMSA on extract from DCs treated with LPS in the presence or absence of 8-Br-cAMP. LPS treatment caused nuclear translocation of NF-κB and induced its DNA binding activity (Fig. 3A, *top panel*). The presence of 8-Br-cAMP did not affect the nuclear translocation and the DNA binding activity of NF-κB induced by LPS. The specificity of the protein-DNA complex was confirmed by a competition assay. Binding was competed by the addition of nonradioactive κB oligonucleotide (data not shown). To investigate further the NF-κB pathway, we monitored the level of the inhibitory subunit IκBα by Western blot. As shown in Fig. 3A (*middle panel*), IκBα protein levels in the whole cell lysate decreased 30 min after treatment with LPS. Such a decrease was not affected by treatment of cells with the cAMP analog 8-Br-cAMP. The reappearance of IκBα 120 min after LPS treatment is a consequence of the NF-κB transcriptional activity. Because 8-Br-cAMP did not impair LPS-induced accumulation of IκBα at later time points, we can conclude that the stimulation of the cAMP-dependent pathway did not affect the transcriptional activity of NF-κB.

LPS has been shown to activate multiple signaling pathways in dendritic cells, including p38 SAPK and ERK (23). Activation of these kinases is associated with their phosphorylation and can be detected by Western blotting using phosphorylation-specific antibodies. We found that the phosphorylated form of p38 SAPK and ERK accumulated within 30 min from the addition of LPS to the cells (Fig. 3B) and remained stable for the next 2 h (Fig. 3B). Stimulation of the cAMP pathway did not prevent these biochemical changes (Fig. 3B). To corroborate this finding further, we measured the phosphotransferase activity of ERK and p38 SAPK on an exogenous substrate. These experiments showed that 8-Br-cAMP increased the stimulation of ERK and p38 activity induced by LPS (Fig. 3C). According to these data, we found that the addition of H89, a selective PKA inhibitor, inhibited accumulation of phospho-ERK induced by LPS (data not shown).

**cAMP Prevents the LPS-induced Activation of c-Src and Lyn Kinases**—A number of studies have documented the ability of the endotoxin to induce accumulation of phosphoryrosine proteins in macrophages (24–26). We therefore measured the autophosphotransferase activity of Src family tyrosine kinases upon stimulation with LPS in the absence or in the presence of the cAMP analog. We found that LPS induced an increase in the activity of c-Src and Lyn which, indeed, was inhibited by the addition of 8-Br-cAMP to the cell culture (Fig. 4).

cAMP and PP2 Exert Comparable Effects on the Endotoxin-mediated Signaling—The role played by the cAMP pathway and Src-like kinases in LPS-induced DC maturation was investigated by comparing the effects induced by 8-Br-cAMP and PP2, a selective inhibitor of Src family tyrosine kinases on LPS-induced cytokine release. In addition, we also evaluated the effects of PGE2, a cyclic nucleoside-elevating agent, on the LPS signaling.

CAMP, PGE2, and PP2 showed a comparable ability to block TNF-α and IL-12 production but exerted the opposite effect on IL-10 secretion, although the cyclic nucleoside and PGE2 stimulated the release of this regulatory cytokine, PP2 blocked it (Fig. 5A). To analyze the molecular basis of this phenomenon, we compared the effects exerted by 8-Br-cAMP, PGE2, and PP2 on the accumulation of transcription factors involved in the regulation of a variety of cytokines genes.

Numerous data have shown the involvement of the interferon regulatory factor 1 (IRF-1) in the modulation of the inflammatory response (27). Recently, the ability of cAMP to regulate IRF-1 synthesis has been documented in murine macrophages (28). Thus, IRF-1 may represent a good candidate to explain the inhibitory effects exerted by 8-Br-cAMP and PP2 on the secretion of IL-12. To test this hypothesis, we measured the level of IRF-1 in DCs exposed to LPS in the presence or absence of 8-Br-cAMP, PGE2, or PP2. IRF-1 was undetectable in iDCs but accumulated after exposure to LPS (Fig. 5B).
biochemical change was markedly impaired by the addition of 8-Br-cAMP, PGE2, or PP2 to the culture media (Fig. 5, B and C).

Signals elicited by endotoxin involve the activation of c-Jun N-terminal kinase that in turn phosphorylates and prevents the degradation of c-Jun (29), a component of the AP-1 complex that regulates the expression of different cytokine genes (30–32). Therefore, we measured the accumulation of c-Jun in DCs that were stimulated with LPS in the presence or absence of 8-Br-cAMP. After 30 min of incubation, c-Src and Lyn were immunoprecipitated, and the auto-phosphotransferase activity was tested on immunocomplexes (IVKA). Western blots (WB) for total c-Src and Lyn are also shown. Results are representative of three independent experiments.

The inhibitory activity of 8-Br-cAMP and PP2 on IL-12 and TNF-α secretion can be explained by the reduced availability of relevant transcription factors such as IRF-1 and c-Jun. On the contrary, the ability of 8-Br-cAMP to stimulate IL-10 synthesis should depend on the accumulation of a set of factors that can be controlled by cAMP as well as by the Src-like kinase-dependent pathway. Because phosphorylation of CREB can be achieved by stimulation of the PKA activity or by a PKA-independent pathway, this molecule represents a good example of this category of transcription factors. Exposure of DCs to LPS induced accumulation of p-CREB, a change that was not inhibited by the addition of 8-Br-cAMP or PGE2 to the medium (Fig. 5, B and C). On the contrary, treatment of iDCs with PP2 completely prevented p-CREB accumulation (Fig. 5B).

On these bases, we propose that Src-like kinases control the accumulation of c-Jun, p-CREB, and IRF-1. The block of this pathway leads to a decrease in the secretion of a variety of cytokines. Otherwise, the impairment of Src-like kinase activations induced by 8-Br-cAMP uncoupled c-Jun and IRF-1 from p-CREB accumulation thereby inhibiting the release of the pro-inflammatory cytokines and favoring the synthesis of IL-10.

**PKA Mediates the Inhibitory Effects Exerted by 8-Br-cAMP on LPS Signaling**—In many cell types elevation of intracellular cAMP levels inhibits cell growth and blocks a number of cellular functions inducing a PKA-dependent phosphorylation of several molecular targets (33). To evaluate the role of PKA in our experimental model, we took advantage of the use of H89, a cell-permeable PKA inhibitor. We found that the addition of H89 to culture media prevented the inhibitory effects exerted by 8-Br-cAMP on the release of cytokines and activation Src family kinases and restored IRF-1 and c-Jun accumulation induced by LPS (Fig. 6, A–C, respectively).

**DISCUSSION**

cAMP represents a second messenger system shared by a variety of hormone receptors, neurotransmitters, and peptide-signaling molecules and is capable of regulating the maturation of dendritic cells. In this report, we show that in the presence of a stable cAMP analog in the cell culture of DCs exposed to LPS does not affect the changes observed in the expression of typical markers that accompany the maturation process of DCs. cAMP displays a remarkable ability to regulate the secretion of pro-inflammatory cytokines in response to LPS which results in the generation of mature DCs that fail to prime the inflammatory response. The analysis of the proximal signaling events triggered by LPS stimulation reveals that cAMP does not block LPS-induced NF-κB and ERK activation, although it enhances p38 MAPK activation. On the contrary, the cyclic nucleoside exerts a marked inhibitory effect on c-Src and Lyn kinases through a PKA-mediated mechanism.

Our findings corroborate previous observations on the ability of cAMP-elevating agents to induce maturation of human monocyte-derived DCs and to favor the phenotypical changes associated with the typical terminal differentiation status induced by LPS (17–19). In this context, we also confirmed the data of la Sala et al. (17) on the inability of cAMP to affect the cell surface level of MHC II molecules. On the contrary, Kam-
bayashi et al. (34) have recently documented a marked reduction of MHC II expression in LPS-stimulated murine bone marrow-derived DCs exposed to 8-Br-cAMP. The mechanisms responsible for the accumulation of MHC II at cell surface level in immature DC exposed to inflammatory stimuli are complex and involve transcriptional as well as post-transcriptional modifications (35, 36). The contribution of these mechanisms as well as their sensitivity to 8-Br-cAMP may vary according to the differentiation stage of DC, their ontogeny, or differences between species. These differences may account for the differential effects reported in the different experimental models.

The signaling pathway triggered by endotoxin engagement of TLR4 is complex and includes several components (37) as follows: the adaptor proteins MyD88 and Mal, the serine-threonine protein kinase interleukin-1 receptor-associated kinases, and the tumor necrosis factor-associated factor 6 (TRAF6) adaptor protein. Stimulation of TLR4 by LPS induces the recruitment of MyD88 and Mal adaptor proteins, interleukin-1 receptor-associated kinase autophosphorylation, and association to TRAF6. Sequentially, TRAF6 induces activation of TAK1 and MKK6. The former, in turn, activates NF-κB, and the latter activates c-Jun N-terminal kinase and p38 MAPK. Little is known about the specific role played by these pathways in the differentiation process of immature DCs. Studies using pharmacological inhibitors demonstrated that the block of p38 SAPK activity prevents the activation of ATF-2 and CREB transcription factors and significantly reduces the LPS-induced up-regulation of CD80, CD83, and CD86 (38). We have found that inhibition of ERK or p38 SAPK results in a marked reduction of LPS-induced TNF-α release (data not shown).

Similarly, the inhibition of NF-κB translocation to the nucleus with an inhibitory peptide was shown to decrease the up-regulation of HLA-DR, as well as that of CD80, CD83, and CD86 (39). It has been shown that the inhibitory effect exerted by IL-10 and vitamin D3 analogs on IL-12 synthesis depends on their capability to interfere with the NF-κB pathway (39, 40).
Therefore, activation of ERK-1/2, p38 SAPK, and NF-κB seems to be crucial in order to sustain the maturation process of DCs. Our data indicate that cAMP can uncouple the release of pro-inflammatory cytokines from the differentiation process without interfering with the activation of these pathways. We therefore propose that the cytokine release and the phenotypical changes associated with the maturation process are under the control of different signaling pathways.

A number of studies (24–26) have documented the ability of the endotoxin to induce the accumulation of phosphotyrosine proteins in macrophages. In this context, it has been also shown that stimulation of human monocytes by LPS rapidly activates CD14-associated Lyn, and the treatment with herbimycin, a tyrosine kinase inhibitor, prevented the production of TNF-α (25). Data from the triple knockout hck–/–, fgr–/–, and lyn–/– macrophages are contrasting; the absence of these kinases did not affect the response to LPS (26). However, even in this murine model, treatment with herbimycin was effective in preventing TNF-α release showing the involvement of an Src-like kinase other than Hck, Fgr, and Lyn in the LPS signaling pathway (26). Little is known on the role played by the Src-like kinases in the activation process of DCs. Recently, the involvement of Lyn in the signaling cascade triggered by stimulation of CD40 receptor has been documented (41). A cross-talk between TRAP proteins and c-Src was found in the signaling cascade triggered by TRANCE, a critical regulator of dendritic cell and osteoclast function, and by IL-1 (42, 43). According to the recent evidence provided by Napolitani et al. (44), our data confirm the role of the Src family tyrosine kinases in the mechanism regulating the release of the cytokines (44). The ability of cAMP to inhibit c-Src and Lyn activity corroborates this hypothesis, identifying for the first time the Src family kinases as targets of the cAMP-dependent pathway in human DCs.

Three main molecular targets of cAMP have been identified as follows: the PKA, the GTP-exchange protein EPAC, and the cyclic nucleotide-gated ion channels (33). Here we show that the inhibitory effects exerted by cAMP on the LPS-induced release of cytokines were completely reverted by the pharmacological block of PKA. Significantly, PKA inhibition also prevented the inhibitory effects exerted by cAMP on the activities of c-Src and Lyn, as well as on LPS-induced IRF-1 accumulation. PKA consists of a complex of two regulatory (R) subunits and two catalytic (C) subunits; the binding of cAMP to the R subunits induces the release of the active C subunits. PKA interferes with many signaling pathways inducing the inactivation of phospholipase C β2, the phosphorylation of a tyrosine phosphatase, the down-regulation of Raf and Rho activities, and the modulation of ion channel permeability (33). In T-cells, PKA activation also determines an increase in the Cak activity (45) which, in turn, results in the inactivation of Lck, an Src family tyrosine kinase, by phosphorylation of the tyrosine residue in its regulatory domain. The ability of cAMP to control the Src family kinases in DCs suggests that, as observed in T-cells, its inhibitory effect may involve the stimulation of Cak by PKA.

Contrasting evidence has been obtained as to whether cAMP-elevating agents are added onto the DCs (17–19). Synergistic effects have been observed on the TNF-mediated signaling and at low doses of LPS (below 10 ng/ml). On the contrary, cAMP-elevating agents inhibit the cytokine release of signal mediated by optimal doses of LPS. These may depend on the complexity of the signal pathway triggered by cAMP (33). Feed-forward or attenuating regulatory mechanisms can be triggered at different intracellular concentrations of the cyclic nucleoside (46). The kinetics of the cAMP level and the availability of protein kinase A-anchoring proteins can also modulate the cAMP signaling (33, 46). In this scenario, changes induced by cAMP on the other receptor-mediated signal pathways may vary according to the type of receptor engaged, as well as to the amount and the biological activity of the offered ligand.

Beside its ability to block the release of TNF-α and IL-12, cAMP also stimulates the release of IL-10, a major regulatory cytokine. The effect of PKA on transcription is mainly achieved by direct phosphorylation of CREB. This change is crucial to allow the interaction of CREB with the transcriptional coactivators CBP and p300 (33). Src-like kinases are able to regulate CREB activation at multiple levels: favoring the accumulation of c-Jun, a factor capable of forming a complex with CREB, and inducing the phosphorylation of CREB by the PKA-independent pathway. Therefore, the quality and the amount of p-CREB complexes could be regulated by the intracellular level of cAMP as well as by the activity of the Src-like kinases. The balance between these biochemical events may control the cytokine profile released by activated DCs.

The cAMP intracellular level is regulated by two types of enzymes: adenylyl cyclase and the cyclic nucleotide phosphodiesterase (33). Nine different adenylyl cyclases are stimulated by interaction with the subunit of Gα proteins. Degradation of cAMP is regulated by at least 12 different members of a large family of phosphodiesterases. The activities of adenylyl cyclases and phosphodiesterases are regulated positively and negatively by other signaling systems, such as calcium signaling (through calmodulin, calmodulin kinase II, calmodulin kinase IV, and calcineurin), subunits of other G proteins (e.g. Gαi, Go, and Gq proteins), inositol lipids (through activation of protein kinase C), and also by tyrosine kinases. This complex scenario suggests that a large number of molecules can be involved in the fine regulation of the intracellular level of the cAMP. Therefore the fine-tuning of this network in the different microenvironments appears to be crucial in modulating the functional plasticity of immature DCs and the final outcome of the immune response.

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