

REVIEW

Regulation of asthma-related T-cell cytokines by the cyclic AMP-dependent signalling pathway

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Introduction

Asthma is a complex disorder characterized by episodic, reversible narrowing of the airways, with associated increased responsiveness of the airways to a variety of specific and nonspecific stimuli. Although the causes of asthma are unknown, an altered activity of cellular functions is a general phenomenon observed in several types of blood cells from asthmatic patients, including eosinophils, monocytes and T cells. The increased activity of T cells is reflected by an increased expression of interleukin (IL)-2 receptor (CD25) and an enhanced production of Th2-like cytokines IL-3, IL-4, IL-5 and granulocyte/macrophage colony-stimulating factor (GM-CSF). Together these cytokines are regarded as the mediators of airway inflammation as observed in asthma patients. A possible explanation for the enhanced cellular activity and the aberrant cytokine gene expression may be an impairment of (a) negative-regulatory signalling system(s). The cyclic-adenosine monophosphate (cAMP)-dependent signalling pathway leading to activation of the protein kinase A (PKA) may be a candidate, since activation of this signalling system results in inhibition of activating signals and abrogation of proliferation of T cells [1–3].

T cells and asthma-related cytokines

T cells are the mediators of cellular immune responses and can be subdivided in CD4+ and CD8+ T-cell subsets. CD8+ T cells mainly eliminate virus-transformed cells and malignant cells, whereas CD4+ T cells regulate the effector functions of immune cells by secretion of cytokines. CD4+ T cells induce inflammatory responses by mobilization of pro-inflammatory immune cells, and support the specific antibody-driven immune response. In the mouse system, T cells are shown to be divided into two well-defined CD4+ T-cell subpopulations, which are discernable on the basis of their cytokine production profiles [4]. Th1 clones produce

IL-2, interferon (IFN)- γ , and tumour-necrosis factor (TNF)- β , whereas Th2 clones produce IL-4, IL-5, IL-6, IL-10 and IL-13. After the discovery of the well-defined Th1 and Th2 clones in the mouse system, a similar dichotomy was proposed for the human system [5]. Human Th2 clones produce a limited panel of cytokines including IL-4 and IL-5. IL-4 induces immunoglobulin-isotype switching in B lymphocytes to IgE production, and may thus be responsible for the observed IgE-mediated early asthmatic reaction. Illustratively, over-expression of IL-4 in transgenic mice resulted in an allergy-like inflammatory disease with enhanced IgE levels [6]. IL-5 has been demonstrated to regulate the production, differentiation, activation and chemoattraction of eosinophils [7]. The prominent role of IL-5 in asthmatic reactions was demonstrated in a mouse asthma model. Knocking out IL-5 gene expression, resulting in IL-5-deficient mice, not only abrogated eosinophil infiltration, but diminished airway hyperresponsiveness and epithelial damage as well [8]. In addition, Th2 cells are capable of producing the cytokines IL-3 and GM-CSF, which are mast cell- and eosinophil-promoting cytokines.

A number of studies have reported abnormalities with respect to T-cell activation in asthmatic subjects. Bronchoalveolar lavage (BAL) and peripheral blood T cells obtained from asthmatics express significantly higher levels of IL-2 receptor (CD25) in comparison with healthy control subjects [9,10]. Expression of other activation markers, i.e. VLA-1 and HLA-DR, were demonstrated to be also enhanced in T cells from asthmatics in comparison with controls [10,11]. In some studies the enhanced expression of these activation markers correlated with increased bronchial hyperresponsiveness. However, not only activation markers are upregulated in T cells from asthmatics. Recent investigations have focused on cytokine patterns of T cells obtained from peripheral blood and/or airways of asthmatic subjects. An enhanced expression of T-cell-derived IL-2, IL-4 and IL-5 mRNA was observed in bronchial biopsies from asthmatics compared with healthy controls [12,13]. Similar observations with IL-4 and IL-5 protein were done in T cells from asthmatics after immunohistochemical staining [14,15]. Additionally, T cells isolated from BAL fluid from asthmatics display higher levels of IL-4 and IL-5

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mRNA and/or protein compared to controls [11,16–18]. An increased number of BAL cells expressing IL-4 and IL-5 mRNA has also been observed 24 h after inhalation of allergen [19,20]. Recent investigations demonstrate that a relationship between IL-5 and severity of asthma disease is becoming increasingly evident [21,22]. IL-4 was not correlated with disease severity but rather to serum IgE levels [22]. These studies suggest that Th2 cell-derived cytokines are responsible for eosinophilia and increased IgE levels observed in the (atopic) asthmatic disease.

The cAMP-dependent signalling pathway

In vivo, the cAMP-dependent signalling pathway can be activated through receptors for prostaglandins of the E type, as well as through β -adrenergic and histamine receptors. These receptors are able to activate cAMP generating enzymes: the adenylyl cyclases. The membrane-bound adenylyl cyclases catalyse the conversion of adenosine triphosphate (ATP) into adenosine-3',5'-cyclic monophosphate (cAMP), which subsequently leads to formation of intracellular cAMP. The binding of cAMP to the regulatory subunit of the tetrameric protein kinase A (PKA) liberates its catalytic subunits, which translocate to the nucleus. Here, the so called cAMP-responsive element binding protein (CREB)—which binds constitutively to cAMP-responsive elements (CRE) in the promoter of cAMP responsive genes—is serine-phosphorylated by the PKA catalytic subunit, which is required to modulate transcription. Many cytokine genes whose expressions are modulated by cAMP, however, lack the CRE in their promoters, for instance IL-2. This implicates an indirect mechanism of action of PKA on gene expression through inhibition of other signal transduction systems. This phenomenon is often referred to as cross-talk between signal transduction systems and the underlying mechanism has long been obscure. Agonists operating via the adenylyl cyclase system that activate PKA were shown to inhibit the PLC tyrosine phosphorylation and suppressed enzymatic activity and antigen-induced phosphatidylinositol turnover [23–25]. An explanation may be that an activated PKA phosphorylates the TcR γ - and ϵ chains, which attenuates TcR signalling [26,27]. Recent studies showed that an activated PKA efficiently phosphorylates Raf-1, and that this resulted in a decreased binding affinity of Raf-1 to p21ras. These latter data suggests that PKA may function to uncouple p21ras from Raf-1 [28].

Soon after the discovery of the cAMP-dependent signalling pathway by Sutherland, Szentivanyi proposed in 1968 a fundamental defect in the adenylyl cyclase system as a possible mechanism leading to asthma [29]. The concept was based on experimental data from rats and mice demonstrating that after administration of Bordetella pertussis

asthma-like symptoms emerged, characterized by hypersensitivity to histamine and nonspecific stimuli (e.g. cold air), eosinophilia and reduced sensitivity to adrenergic agonists. This hypothesis led to a great number of studies to investigate the role of adenylyl cyclase in asthma. From these studies it was concluded that the AC system of peripheral blood mononuclear cells (PBMC) obtained from asthmatic patients in a stable phase of their disease (defined as having no history of respiratory tract infections or acute asthmatic attacks for at least 2 months) displays normal activities of adenylyl cyclase in comparison with healthy persons [30–34]. However, 24 h after an allergen-induced airway obstruction, a 40–50% impairment of adenylyl cyclase activity was observed. Simultaneously, an increase of bronchial hyperresponsiveness in these patients occurred. More recently, it was demonstrated that bronchial hyperresponsiveness and the impairment of adenylyl cyclase in asthmatic patients are already present 3 h after allergen provocation [34]. It has also been demonstrated that in normal PBMC the activity of the adenylyl cyclase system could be potentiated by cell-activating stimuli, which themselves do not activate the adenylyl cyclase system, e.g. phytohaemagglutinin, phorbol esters or Ca^{2+} – ionophores [35]. In contrast, PBMC obtained from asthmatic subjects in a stable phase of the disease lacked this potentiation completely or partially. The extent of lack of potentiation was positively correlated with bronchial hyperresponsiveness [36]. The observation that PBMCs from asthma patients have diminished intracellular cAMP levels after allergen challenge together with an impaired potentiation of the adenylyl cyclase system in these cells, may have consequences for the expression of cytokines that are regulated by PKA.

Regulation of asthma-related T-cell cytokines by cAMP

At present it is thought that activation of the cAMP-dependent signalling pathway may contribute to the generation of Th2 lymphocytes, since it has been shown that this pathway provides a strong inhibitory signal for Th1 cytokines, whereas Th2 cytokines are not affected or even upregulated by this signal transduction system. IL-4 mRNA induced by phorbol-myristate acetate (PMA)—a direct activator of protein kinase C (PKC)—plus the calcium ionophore A23187 in T-cell lines did not respond to elevations of cAMP, whereas IL-2 mRNA was completely inhibited [37]. Moreover, forskolin and cholera toxin, both inducers of intracellular cAMP, could not modulate IL-4 mRNA expression in T cell lines stimulated with antibodies against CD3 [38,39]. In this respect PGE2 effects on cytokine gene expression is also of relevance, since T cells have ample opportunity to contact PGE-secreting cells. In the migratory process of T cells from the circulation towards the lung

several types of PGE-producing cells might be contacted: macrophages, fibroblasts, dendritic cells and epithelial cells. The productions of IFN γ and IL-2 from Th1 clones were inhibited by PGE2. In contrast, IL-4 productions from Th2 and Th0 clones were not affected, whereas IL-5 production from Th2 clones was upregulated by PGE2 [39,40]. Priming of CD4 + T cells with PGE2 inhibited the production of IL-2 and IFN γ , leaving IL-4 and IL-5 productions unaffected [41]. In addition, in the murine thymoma cell line EL-4 it has been shown that IL-5 gene expression is induced by PMA alone, which could be synergistically upregulated by dibutyryl-cAMP, a cAMP analogue [42].

These observations on T cell clones and T cell lines are in contrast with our data in freshly isolated human T cells. Recently, we provided evidence that the effect of cAMP elevation on IL-4 and IL-5 cytokine gene expression was highly subject to the external stimulus. In particular, the PGE2-induced inhibitory effect on IL-4 gene expression observed in anti-CD3 plus anti-CD28-activated T cells was not observed in cells stimulated with PMA plus a calcium-ionophore [43]. Additionally, PGE2-inhibited IL-5 gene expression in anti-CD3 plus anti-CD28-activated T cells, whereas PGE2 upregulated IL-5 in the presence of PMA plus Con A [44]. Similar effects were demonstrated for IL-3 and GM-CSF gene expression, albeit less pronounced [45]. Hence, the inhibitory effect of PGE2 on Th2-like cytokines appears to be abrogated by direct activation of the protein kinase C (PKC) by PMA. Since it has been observed that PKA activation inhibits ligand-stimulated phosphatidylinositol (PI) metabolism [46], it can be reasoned that PKA activation inhibits the nuclear factor of activated T cells (NF-AT) and NF- κ B. These transcription factors are likely to be involved in Th2-like cytokine gene expression. We showed that NF-AT binding to DNA is not affected by cAMP elevations, while NF- κ B is completely blocked [44]. In addition, PKA activation abrogates tyrosine phosphorylation of p21ras. The concomitant activation of PKC can overcome the inhibition on tyrosine phosphorylation of p21ras, while it did not antagonize the uncoupling of the T cell receptor from PI metabolism [47]. Alternatively, PKC is able to phosphorylate sphingosine kinase, leading to activation of sphingosine-1-phosphatase, an activator of Raf-1 independent of p21ras [48]. Sphingosine kinase is also able to activate the stress activated protein kinase (SAPK) via a unclear mechanism, but probably through the phosphorylation of MEKK-1. These data demonstrate that the concomitant PKC activation bypasses the inhibitory effects of PKA as a result of the activation of several signalling pathways (Fig. 1). In addition, direct activation of the PKC by PMA may also induce Th2-like mRNA stabilization, as has been demonstrated for IL-3 and GM-CSF mRNA transcripts [45]. Together these mechanisms of action of PMA may result in an enhanced expression of

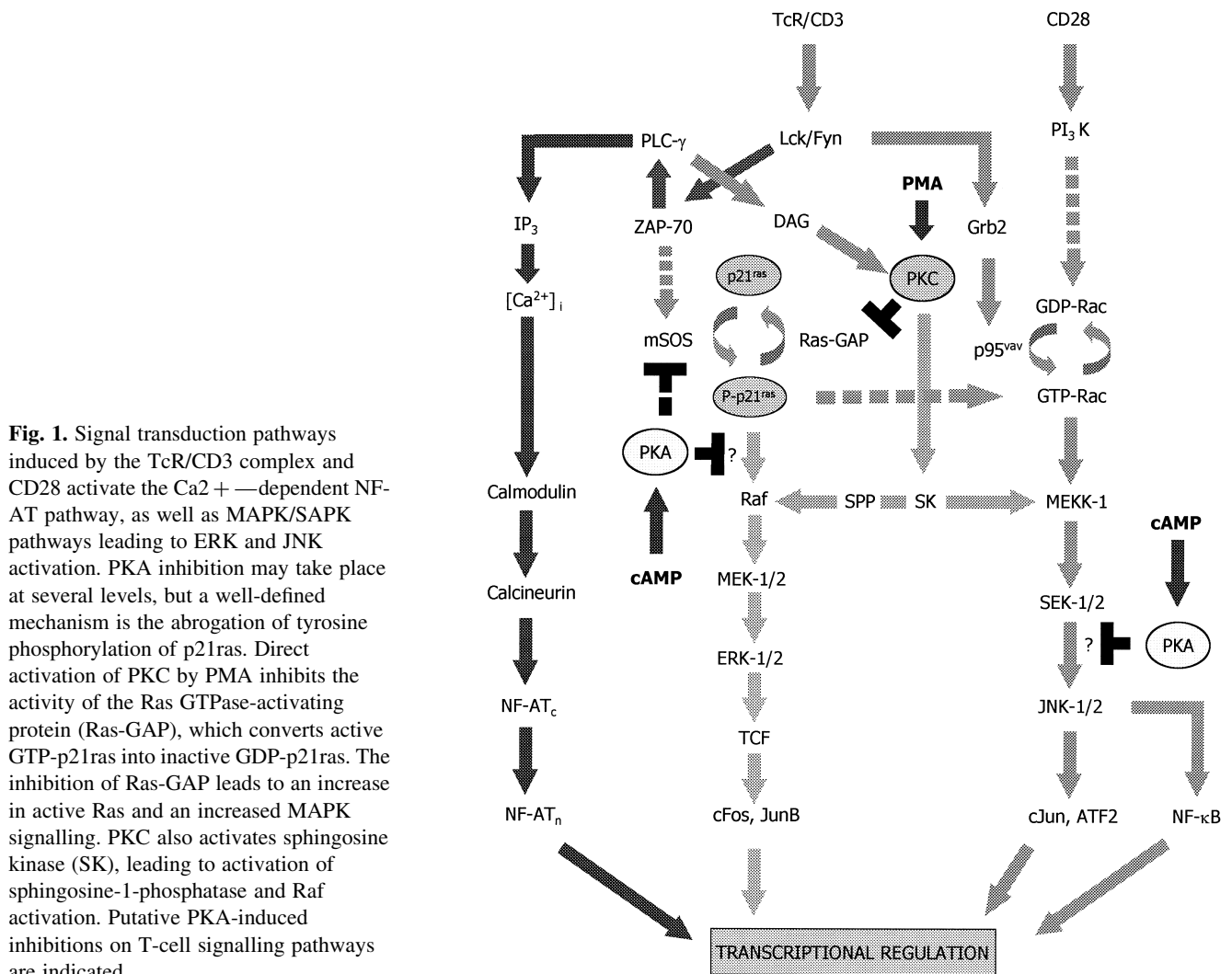
Th2-like cytokines: PKC activation bypasses the PKA-mediated inhibition through activation of additional signalling pathway(s) and reduces the decay of cytokine messages. The precise mechanism(s), however, still has to be elucidated.

Similar cross-regulating mechanisms may be relevant *in vivo*, since cytokines have an inhibitory effect on cAMP generation by adenylyl cyclases. A cytokine-containing medium (including IL-1, IL-2) was able to reduce β -adrenergic receptor-induced cAMP accumulation in myocardial cells by uncoupling adenylyl cyclase and G-proteins [49]. We demonstrated that PGE2-induced inhibition on IL-5 protein secretion could also be overridden by the addition of IL-2, IL-7 or IL-15 [44]. Although these cytokines induce the same signal transducer and activator of transcription proteins (Stat5) it is not clear whether they interact with the cAMP dependent signalling pathway. A comparable mechanism has been described for IL-4 and IL-5 production by Th0 clones. In the presence of high concentrations of IL-2, secretion of IL-4 and IL-5 protein were enhanced by PGE2. In contrast, when IL-2 concentrations during incubation were low IL-4 and IL-5 protein secretions were inhibited [50].

T-cell cytokine gene expression in asthma

The observation that the mode of stimulation determines the ultimate effect of the cAMP-dependent signalling pathway on cytokine gene expression may be of relevance in asthmatic patients. Activation marker expressions on T cells from asthma patients are enhanced in comparison with healthy control subjects, indicating that these cells have a higher degree of activity [9,10]. An enhanced activation state of the asthmatic T cell, due to diminished internal feedback control by the cAMP-dependent signalling pathway, may theoretically lead to enhanced expression of Th2-like cytokines [43–45]. In agreement with several publications, we recently demonstrated that IL-4 and IL-5 mRNA levels are increased in activated peripheral blood T cells obtained from asthmatic patients and this may reflect the increased cellular activity. Although IFN γ was more prone to PGE2 inhibition than IL-4, both asthma patients and healthy controls were shown to be equally sensitive for PGE2 inhibitions with respect to IFN γ and IL-4 gene expression [51].

β -Adrenoceptor signalling behaves completely different from PGE2 receptors. Before allergen provocation (AP), β -adrenoceptor stimulation modulated the expression of both IFN γ (down) and IL-5 (up) (Fig. 2). Surprisingly, 24 h after AP we found a complete desensitization of β -adrenoceptor signalling with respect to IFN γ and IL-5 modulation [52], while only a 40–50% effect would have been expected [31–33]. It has been shown that Ca²⁺ – dependent isotype(s) of adenylyl cyclases are involved in the mechanism of



potentiation of cAMP generation [36]. Complete loss of β -adrenoceptor-mediated signalling therefore may be due to desensitization of these Ca^{2+} —dependent adenylyl cyclases (type I and III) which are also responsible for the observed modulation on cytokine gene expression. PGE₂-induced inhibitions on cytokine gene expression were similar both before and after allergen challenge, suggesting the involvement of Ca^{2+} —independent adenylyl cyclases [52]. These data may also implicate that an impaired potentiation of PGE₂-coupled adenylyl cyclase does not further deteriorate after AP, which is the case for β -adrenoceptor-coupled adenylyl cyclases.

Moreover, 24 h after AP peripheral blood T cells from asthma patients become 'primed' (i.e. enhanced activity) to produce increased IL-5 mRNA levels, whereas IFN γ mRNA levels are similar both before and after AP [52]. The underlying mechanism leading to enhanced IL-5 mRNA therefore seems to be related to presence of allergen.

Permanent exposure of asthma patients to low levels of allergen may also be reflected in the enhanced expression of Th2-like cytokines in BAL and/or peripheral blood. It is tempting to speculate that the presence of allergen leads to the 'primed' state of the T cells as a result of diminished negative feedback control normally mediated by the cAMP-dependent signalling pathway. How the allergen may induce the primed state of T cells is not clear, but several allergens have proteolytic activity (e.g. Der p 1) that may 'prime' the T cells via protease-activated receptors. Alternatively, soluble mediators released during the inflammatory response in the lung possibly leak into the circulation and prime the T lymphocytes for enhanced IL-5 productions. Recently, a similar mechanism has been proposed for the release of haemopoietic progenitor cells into the periphery of atopic patients: release of locally-produced mediators into the blood circulation are responsible for the increased levels of progenitors [53]. The character of the putative mediators

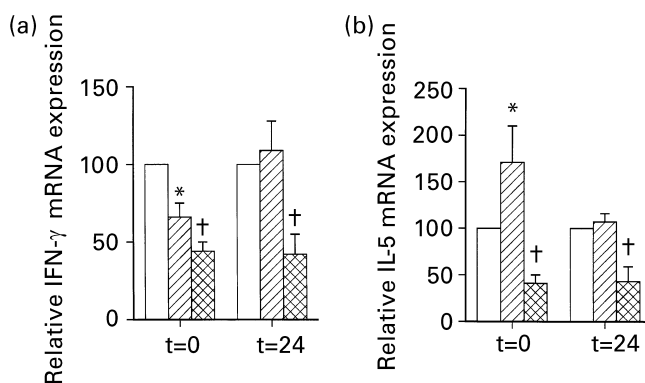


Fig. 2. Effect of two activators of the PKA on IFN γ and IL-5 mRNA expression in T cells isolated from peripheral blood obtained from patients with asthma, before (t = 0) and 24 h after allergen provocation (t = 24). T cells were activated with antibodies against CD3 plus CD28 in the presence of 10 mM isoprenaline (hatched bars) and 10 mM PGE2 (double hatched bars). Before and after allergen provocation, PGE2 firmly downregulated both IFN γ and IL-5 mRNA. The modulatory effect of isoprenaline on IFN γ (down) and IL-5 mRNA (up) observed before allergen provocation completely disappeared 24 h after allergen provocation. Relative mRNA levels are expressed as the percentage of initial mRNA levels in the absence of PKA activator (100%). * $P < 0.05$ vs in absence of ISO. † $P < 0.03$ vs in absence of PGE2.

are at present not known, but candidates may be PGE2 in combination with cytokines using the common IL-2 receptor γ -chain, since it was demonstrated that in the presence of these cytokines PGE2 upregulated the expression of IL-4 and IL-5 [45,50].

Concluding remarks and discussion

Balanced expression of Th1- and Th2-like cytokines requires a very complex network of regulatory events. The cAMP-dependent signalling pathway leading to PKA activation provides an inhibitory signal for the expression of both Th1- and Th2-like cytokines. The ultimate effect of PKA activation on Th2-like cytokines, however, seems to be highly dependent on the microenvironment of the T cells. For IL-4 and IL-5 this has clearly been demonstrated in freshly isolated human T cells, where the effect of PGE2 was dependent on the mode of stimulation and on the presence of cytokines using the IL-2 receptor γ -chain. In the absence of these cytokines PGE2 exerts an inhibitory effect on the expression of both IL-4 and IL-5. Most published observations on IL-4 and IL-5 gene expression have been performed in T cell lines and T cell clones. Distinct intracellular signalling of T cell lines compared with freshly isolated T cells could provide an explanation for the discrepancy between our results and the results found in cell lines. This view is supported by our unpublished

observation that PMA alone cannot induce cytokine gene expression in freshly isolated T cells, which is a phenomenon found in T cell lines [41]. Allergen-specific Th2 clones may also have a higher degree of activity due to allergen-binding, which may result in polarized cells still producing IL-4 and IL-5, but not IFN γ , in response to PGE2. Alternatively, the polarization may result from an impaired cAMP signalling in allergen-specific Th2 clones obtained from asthmatics due to allergen-induced desensitization of adenylyl cyclases. Besides, a diminished lung inflammation observed in asthma patients after inhalation of PGE2 [54] may also be explained by the inhibition of Th2-like pro-inflammatory cytokines, since a PGE2-induced upregulation of Th2-like cytokines would theoretically lead to enhanced inflammation.

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