The cGMP/Protein Kinase G Pathway Contributes to Dihydropyridine-sensitive Calcium Response and Cytokine Production in TH2 Lymphocytes*

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Th2 lymphocytes differ from other CD4+ T lymphocytes not only by their effector tasks but also by their T cell receptor (TCR)-dependent signaling pathways. We previously showed that dihydropyridine receptors (DHPR) involved in TCR-induced calcium inflow were selectively expressed in Th2 cells. In this report, we studied whether cGMP-dependent protein kinase G (PKG) activation was implicated in the regulation of DHPR-dependent calcium response and cytokine production in Th2 lymphocytes. The contribution of cGMP in Th2 signaling was supported by the following results: 1) TCR activation elicited cGMP production, which triggered calcium increase responsible for nuclear factor of activated T cell translocation and IL4 gene expression; 2) guanylate cyclase activation by nitric oxide donors increased intracellular cGMP concentration and induced calcium inflow and IL-4 production; 3) reciprocally, guanylate cyclase inhibition reduced calcium response and Th2 cytokine production associated with TCR activation. In addition, DHPR blockade abolished cGMP-induced [Ca2+]i increase, indicating that TCR-induced DHP-sensitive calcium inflow is dependent on cGMP in Th2 cells. Th2 lymphocytes from PKG1-deficient mice displayed impaired calcium signaling and IL-4 production, as did wild-type Th2 cells treated with PKG inhibitors. Altogether, our data indicate that, in Th2 cells, cGMP is produced upon TCR engagement and activates PKG, which controls DHP-sensitive calcium inflow and Th2 cytokine production.

T-helper cells of type 2 (Th2) constitute a distinct subset of CD4+ T lymphocytes (1) involved in the elimination of extracellular pathogens (2). However, exacerbated Th2 responses are also associated with allergic manifestations, including asthma. Specific signaling pathways are dedicated to cytokine production by Th2 cells (3–8). Calcium-dependent signaling pathway is necessary and sufficient for IL4 gene expression. Transfection of non-IL-4–producing T lymphocytes with constitutively active calcineurin induces IL4 gene promoter activity (9, 10). Calcium-independent pathways also contribute to Th2 cell differentiation and IL-4 production upon T cell receptor (TCR)3 stimulation. For example, the mitogen-activated protein kinase extracellular signal-regulated kinase (ERK) facilitates Gata-3-mediated chromatin remodeling at Th2 cytokine loci (11).

TCR engagement results in phosphorylation events and scaffolding of various adapters and enzymes. This TCR signaling complex, called sigmalsome, integrates signaling pathways into transcriptional events (12). One of these pathways is orchestrated by the second messenger calcium. Activated phospholipase Cγ1 cleaves phosphatidylinositol (4, 5)-biphosphate into inositol (1, 4, 5)-triphosphate (IP3) and diacylglycerol (DAG). DAG leads to protein kinase C and Ras-dependent mitogen-activated protein kinase activation (13), whereas IP3 mobilizes intracellular calcium stores. Calcium mobilization is followed by a calcium inflow through calcium release-activated calcium (CRAC) channels (reviewed in Ref. 14) and subsequent activation of the nuclear factor of activated T cells (NFAT). Once dephosphorylated by calcineurin, a calcium-activated phosphatase, NFAT translocates to the nucleus and binds together with other transcription factors to target genes, including cytokine genes (discussed in Ref. 12).

Intracellular calcium concentration was reported to be higher in resting Th2 lymphocytes than in other T cell subsets. Only low calcium concentration increase was measured in activated Th2 lymphocytes (3–5, 15–17), suggesting an original [Ca2+]i regulation mechanism in Th2 lymphocytes. We have previously shown that in Th2 cells TCR engagement involved dihydropyridine receptor (DHPR)-dependent calcium inflow (18). DHPR were also detected in other immune cells including natural killer (NK) cells (19), dendritic cells (20), B lymphocytes (21, 22), and naive T cells (23, 24). In B cells, DHPR-dependent [Ca2+]i increase subsequent to antigen receptor stimulation was shown to be mediated by cGMP (21).

In this study, we wondered whether cGMP was involved in the control of calcium response and cytokine production in Th2 lymphocytes. Our data show that TCR-dependent cGMP production results in PKG activation, which promotes DHP-sensitive calcium inflow and IL-4 synthesis.

EXPERIMENTAL PROCEDURES

Animals and Reagents—7–12-week-old OVA (323–339)-specific DO11.10 TCR transgenic BALB/c mice (25), BALB/c mice (January

The abbreviations used are: TCR, T cell receptor; Ab, antibody; B-Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; B-Br-cGMP, 8-bromoguanosine 3′,5′-cyclic monophosphate; DHPR, dihydropyridine; DHPR, DHP receptor; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; mAb, monoclonal antibody; NFAT, nuclear factor of activated T cell; NO, nitric oxide; NONOate, spermine NONOate; PDE, phosphodiesterase; PKG, cGMP-dependent protein kinase; SNP, sodium nitroprusside; NK, natural killer; HPRT, hypoxanthine phosphoribosyl transferase.

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Ets, Le Genest St. Isle, France), and 4–6-week-old PKG1-deficient (PKG1−/−) mice (26) on a 129/Sv genetic background were cared for in the animal facility of the Regional Committee on animal experimentation. All aspects of animal care were approved by our institutional review board for animal experimentation. The calcium channel antagonist R(+)-Bay K 8644, sodium nitroprusside (SNP), 8-bromoguanosine 3′:5′-cyclic monophosphate (8-Br-cGMP), and 8-bromoadenosine 3′:5′-cyclic monophosphate (8-Br-cAMP) were purchased from Sigma. The hamster anti-mouse TCR H57–597 mAb (27) was from BD Biosciences. Nicardipine was from Novartis (Basel, Switzerland).

IL-4 mRNA Quantification—Total RNA was isolated from 5 × 10^6 cells using the SV total RNA isolation system (Promege, Madison, WI) and reverse transcribed to cDNA using poly(dT) and Moloney murine leukemia virus reverse transcriptase (Invitrogen). IL-4 and hypoxanthine phosphoribosyl transferase (HPRT) mRNA were measured by real-time quantitative PCR using an ABI Prism 7000 sequence detection system. PCR was performed with the PCR SYBR Green sequence detection system (PerkinElmer). Primers for IL-4 were 5′-CGGTGAACGTAGGAAAACTCTGTAG-3′ and 5′-CAGGGTGACGTCTTCAGTGTG-3′ and for HPRT 5′-CTGGTGAAGAAAGCCTTCG-3′ and 5′-TGAGACTACTTATGATCAGGCGA-3′. IL-4 mRNA was normalized to HPRT mRNA and quantified relative to mRNA expression in unstimulated Th2 cells.

**Flow Cytometric Analysis of IL-4 and IL-5 Intracellular Cytokine Synthesis**—Th2 cells were stimulated on plate-bound anti-TCR mAb in the absence or in the presence of either nicardipine or LY-83,583 (10 μM). After 12 h of culture, cells were collected, resuspended at 10^6/ml, and stimulated with phorbol 12-myristate 13-acetate (50 ng/ml, Sigma) + ionomycin (0.5 μg/ml, Sigma) for 4 h. Two hours before cell harvest, 10 μg/ml of brefeldin A (Sigma) was added. Cells were then fixed with 4% paraformaldehyde (Fluka Chemie, Buchs, Switzerland). Intracytoplasmic staining was performed as previously described (33). Briefly, after washing and 10 min of incubation in saponin medium alone, cells were incubated with phycoerythrin-conjugated rat anti-mouse IL-4 (11B11) or anti-mouse IL-5 (TRFK5) antibodies (BD Biosciences) for 30 min at 4°C. Cells were washed first in saponin buffer and then with phosphate-buffered saline to allow membrane closure. Data were collected on 20,000 CD4+ cells on an XL Coulter cytometer (Coultronics, Margency, France) and analyzed using the CellQuest software (BD Biosciences).

**PCR Analysis**—PCR conditions were 94°C for 45 s, 60°C for 45 s, and 72°C for 1 min for 21 (β actin) or 40 cycles (for PKG1). Primers for β actin were 5′-TGGAAATCCCTGTGACCGCTATCAGAAAC and 5′-TAAAGCAGCCTAGTAAAGCTC and for PKG1 were 5′-ATGGTAGTGTACCCCGTGGAAT and 5′-TTGGTGAAGTCTTCTCGGTTA.

**Western Blot Analysis**—5–10^6 cells were lysed for 15 min in phosphate-buffered saline containing 4 mM EDTA, 1% Triton, 150 mM NaCl, 20 mM Tris-HCl, pH 8, protease inhibitor mixture (1 tablet/6 ml of buffer (Roche Diagnostics), aprotinin, leupeptin, and pepstatin (1 μg/ml each), 1 mM phenylmethylsulfonyl fluoride, 5 mM tetrasodium pyrophosphate, 1 mM orthovanadate sodium, and 1 mM NaF. Lysates were spun at 13,000 rpm to remove insoluble material. Protein content was measured using a detergent-compatible protein assay (Bio-Rad). Samples (40 μg/lane) were subjected to 7.5% SDS-PAGE and transferred onto nitrocellulose membranes (Bio-Rad). Membranes were saturated and incubated overnight with anti-PKG1α/β Ab (Santa-Cruz). Blots were developed with a rabbit anti-goat Ab and then a horseradish peroxidase-conjugated anti-rabbit Ab and the enhanced chemiluminescent system (Amersham Biosciences).

**Statistical Analysis**—Results are expressed as the mean ± S.D. Overall differences between variables were initially shown by using a Kruskal-Wallis test and subsequently confirmed by the Mann-Whitney U test.
RESULTS

cGMP Induces a Calcium Response, NFAT Translocation to the Nucleus, and IL4 Gene Expression in Th2 Lymphocytes—In B lymphocyte, cGMP was shown to be responsible for DHPR activation and subsequent calcium influx upon B cell receptor stimulation (21). Because we have recently shown that Th2 cells expressed DHPR (18), we investigated the role of cGMP in TCR-induced DHP-sensitive calcium response in these cells. DO11.10 cells were differentiated along the Th2 pathway by three weekly stimulations with antigen-presenting cells loaded with the OVA 323–339 peptide in the presence of IL-4 and were loaded with 5 μM Fura-2AM. Basal intracellular calcium concentration was recorded at the single cell level for 60 s. The arrow indicates the addition of either 8-Br-cGMP (200 μM) or 8-Br-cAMP (200 μM). Each curve represents the mean of 25–40 cells analyzed during one experiment and is representative of four independent experiments. B, Th2 cells were cultured with 8-Br-cGMP (200 μM) for 4 h. They were then stained with anti-NFATc1 antibody. Unstimulated or stimulated with plate-bound anti-TCR mAb (1 μg/ml) overnight. They were then washed and loaded with 5 μM Fura-2AM. Basal intracellular calcium concentration was recorded at the single cell level for 60 s. The arrow indicates the addition of either 8-Br-cGMP (200 μM) or 8-Br-cAMP (200 μM). Each curve represents the mean of 25–40 cells analyzed during one experiment and is representative of four independent experiments. C, Th2 cells were cultured with 8-Br-cGMP (200 μM) for 4 h. They were then stained with anti-NFATc1 antibody. Unstimulated or stimulated with plate-bound anti-TCR + anti-CD28 mAbs for 4 h were used as negative and positive controls, respectively. D, the histogram summarizes, for all cells within each field examined, the ratios between nuclear and cytoplasmic NFAT fluorescence (mean ± S.E., at least 100 cells for each condition; *, p < 0.01 when compared with unstimulated cells). E, Th2 cells were incubated for 6 h with 8-Br-cGMP (200 μM) in the presence or in the absence of cyclosporin A (CsA, 0.1 μg/ml). Th2 cells stimulated on plate-bound anti-TCR mAb (1 μg/ml) were used as positive control. IL-4 mRNA was quantified by real-time quantitative PCR and normalized to HPRT mRNA. Values were standardized relative to mRNA levels in unstimulated Th2 cells. Results are expressed as mean ± S.D. of two experiments performed in triplicate (**, p < 0.01; ***, p < 0.005).

8-Br-cAMP, another cyclic nucleotide known to promote Th2 cytokine production, had no effect on Th2 cell calcium response (Fig. 1B).

We then wondered whether the 8-Br-cGMP-induced [Ca2+]i increase was sufficient to induce NFAT translocation to the nucleus. Th2 cells incubated for 4 h in the presence of 8-Br-cGMP exhibited a significant NFAT translocation as shown by confocal microscopy (Fig. 1, C and D). Depending on the experiments, NFAT was found to be translocated to the nucleus in 2–11% of resting Th2 lymphocytes, 30–50% of cGMP-treated cells, and 41–75% of anti-CD3 + anti-CD28-stimulated lymphocytes (not shown). Because IL4 gene expression is regulated by the calcium-dependent calcineurin pathway (9), we assessed whether cGMP triggered IL4 gene expression. 8-Br-cGMP induced a strong IL-4 mRNA increase. The inhibitory effect of cyclosporin A testified to the involvement of calcium–calcineurin pathway (Fig. 1E). Contrasting with TCR-stimulated Th2 cells, IL-4 was not detected at the protein level in supernatants collected 24 h after cGMP stimulation (not shown). The lower calcium response (Fig. 1, C and D) and IL4 gene expression (Fig. 1E) induced by cGMP could account for this difference.

An Inhibitor of Guanylate Cyclases, LY-83,583 Strongly Reduces Calcium Response and Type-2 Cytokine Production upon TCR Stimulation—[Ca2+]i increase induced by stimulation of Th2 lymphocytes with a combination of both anti-TCR and anti-CD28 mAbs was abolished by LY-83,583 (Fig. 2A). The calcium response was also inhibited either in the absence of extracellular calcium or in the presence of a DHPR antag-
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FIGURE 3. NO donors induce cGMP production, calcium response, and IL-4 expression in Th2 lymphocytes. A, D011.10 Th2 cells were stimulated for 5 min either on plate-bound anti-TCR mAb (1 μg/ml), sodium nitroprusside (SNP, 150 μM), or NONOate (300 μM) in the presence or in the absence of the PDE5 inhibitor zaprinast (10 μM). Cells were lysed, and intracellular cGMP content was determined by ELISA and normalized to total protein contents (p < 0.001 when compared with unstimulated cells). B, modulation was assessed of intracellular calcium concentration by NONOate or SNP (arrow, 300 and 150 μM, respectively) in Th2 cells pretreated or not with nicardipine or LY-83,583 (10 μM). 3 mM EGTA-containing extracellular medium was designated [Ca2+]ext. Each curve represents the mean of 25–40 cells analyzed during one experiment and is representative of four independent experiments. C, Th2 cells were incubated with SNP (150 μM) for 6 h in the presence or in the absence of cAMP (A, 0.1 μM). Th2 cells stimulated on plate-bound anti-TCR mAb (1 μg/ml) were used as a positive control. IL-4 mRNA was quantified by real-time PCR and normalized to HPRT mRNA. Values were standardized relative to mRNA levels in unstimulated Th2 cells. Values are expressed as mean ± S.D. of two experiments performed in triplicate (p < 0.001; *p < 0.005). D, Th2 lymphocytes were cultured with NONOate (1 μM) for 4 h and stained with anti-NFAT1 antibody. The histogram summarizes, for all cells within each field examined, the ratios between nuclear and cytoplasmatic NFAT fluorescence (mean ± S.E., at least 100 cells for each condition, three experiments performed in duplicate; *, p < 0.01 when compared with unstimulated cells). E, Th2 cells from PKG1−/− and +/− littermates were stimulated on plate-bound anti-TCR mAba (1 μg/ml) for 24 h, and IL-4 content was determined in supernatant by ELISA. Values are expressed as mean ± S.D. of three experiments (from three litters comprising 6 PKG+/− mice) performed in triplicate (*, p < 0.001).

FIGURE 4. PKG1−/− Th2 lymphocytes display impaired calcium response and IL-4 production. A, CONA of 3-week stimulated Th2 lymphocytes from PKG1−/− and +/− mice were amplified with PKG1- and β actin-specific primers. B, lysates of PKG1−/− and +/− Th2 cells were immunoblotted with anti-PKG1/β Ab. C, intracellular calcium concentration was measured in PKG1−/− and +/− Th2 cells loaded with Fura-2-AM and stimulated with anti-TCR (1 μg/ml) + anti-CD28 (1 μg/ml) mAbs (arrow). Each curve represents the mean of at least 40 cells and is representative of cells differentiated from 3 mice/group and tested in duplicate. D, NFAT translocation was assessed in PKG1−/− and +/− Th2 lymphocytes stimulated on plate-bound anti-TCR + anti-CD28 mAbs for 4 h. The histogram summarizes, for each mouse, the ratios between nuclear and cytoplasmatic NFAT fluorescence (mean ± S.E., at least 100 cells for each condition, *p < 0.01 when compared with unstimulated cells). E, Th2 lymphocytes from PKG1−/− and +/− littermates were stimulated on plate-bound anti-TCR mAba (1 μg/ml) for 24 h, and IL-4 content was determined in supernatant by ELISA. Values are expressed as mean ± S.D. of three experiments (from three litters comprising 6 PKG+/− versus 8 PKG−/− mice) performed in triplicate (*p < 0.001).

onist (nicardipine) (Fig. 2A). Data were confirmed with another DHP antagonist, R(+) BayK 8644 (not shown). Treatment of TCR-activated Th2 cells with LY-83,583 as well as nicardipine resulted in dose-dependent inhibition of IL-4 (Fig. 2B) and IL-5 (Fig. 2C) release. Intracellular IL-4 and IL-5 staining revealed that LY-83,583 and nicardipine dramatically reduced the number of IL-4- and IL-5-producing cells (Fig. 2D).

NO Donors Generate cGMP, Increase [Ca2+]i, Induce NFAT Nuclear Translocation and IL-4 Production—The role of guanylate cyclases was further examined by using NO donors that activate soluble guanylate cyclases (34). SNP induced a 2-fold increase in intracellular cGMP concentration, in the same range as TCR stimulation did (Fig. 3A). NONOate, a more potent NO donor than SNP, induced a 10-fold increase in intracellular cGMP concentration when compared with unstimulated cells (Fig. 3A). Zaprinast, an inhibitor of phosphodiesterase 5 (known to specifically promote cGMP degradation), did not enhance cGMP production, indicating that cGMP degradation did not minimize cGMP production (Fig. 3A). Both NO donors, SNP and NONOate, triggered a sustained [Ca2+]i increase (Fig. 3B). However, the calcium response induced by NONOate was more abrupt and higher than the one induced by SNP. The guanylate cyclase inhibitor LY-83,583 delayed and reduced SNP-induced calcium response (Fig. 3B). The SNP-induced [Ca2+]i increase was inhibited either in the absence of extracellular calcium or in the presence of DHPR antagonist nicardipine (Fig. 3B). SNP induced Il4 gene expression, which was abolished in the presence of cyclosporine A (Fig. 3C). We failed to detect any SNP-induced IL-4 protein secretion (not shown). According to its high potency to enhance cGMP production, NONOate induced NFAT nuclear translocation (Fig. 3D) in 60–70% of cells (not shown) and detectable IL-4 production (Fig. 3E). By contrast, the dose-dependent increase in IL-4 production triggered by NONOate was not observed in Th2 cells from PKG1−/− mice, suggesting a prominent role of PKG in mediating NONOate effect (Fig. 3E). Altogether, these data showed that guanylate cyclase activation resulted in cGMP production and a subsequent DHP-sensitive extracellular calcium inflow, responsible for IL-4 expression.

Calcium Signaling and IL-4 Secretion Are Defective in Th2 cells from PKG Null Mice—PKG or cyclic nucleotide phosphodiesterases (PDE) are putative targets for cGMP. In turn, PDE enzymes regulate cAMP and cGMP levels. As shown in Fig. 4A, PKG1 mRNA was found in Th2 lymphocytes. PCR specificity was demonstrated by the absence of amplification in Th2 cells from PKG1−/− mice. PKG1 protein was detected in PKG1+/− but not in PKG−/− Th2 cells (Fig. 4B). Then we investigated the involvement of PKG in TCR-induced calcium response and subsequent IL-4 production. CD4+ T cells from PKG1−/− mice and PKG1−/− littermates were differentiated along the Th2 pathway by three weekly polyclonal stimulations in the presence of IL-4 + anti-interferon γ antibody. Calcium response in PKG1−/− Th2 lymphocytes was dramatically reduced as compared with PKG1+/− Th2 cells (Fig. 4C). NFAT nuclear translocation was also diminished in Th2 lymphocytes from PKG1−/− mice (Fig. 4D). Only 8–25% of PKG1−/− versus 60–78% of PKG1+/− Th2 cells displayed NFAT nuclear translocation.
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**FIGURE 6.** PKG inhibitor does not modify IL-4 synthesis in PKG-deficient Th2 lymphocytes. Th2 lymphocytes from PKG1−/− and ++/− littermates (from a litter different from those used in Fig. 4E) were stimulated on plate-bound anti-TCR mAb. Cultures were done in the presence or in the absence of Rp-8-pCPT-cGMPS (200 μM), 24 h later, IL-4 content was determined in supernatants. Stimulation assays were performed in triplicates. The experiment includes three mice per group. Values are expressed as mean ± S.D. (*, p < 0.01 when compared with the wild-type group in the absence of inhibitor).

**DISCUSSION**

Our results strongly support a pivotal role for cGMP and PKG in calcium signaling and IL-4 synthesis in Th2 lymphocytes. Contribution of cGMP in Th2 signaling was underlined by the following data: 1) cell-permeant cGMP, or nitric oxide donors known to activate soluble guanylate cyclase, triggered a calcium response and IL-4 expression; 2) addition of guanylate cyclase inhibitor LY-83,583 suppressed both [Ca^{2+}]_i, increase and Th2 cytokine production induced by TCR activation. Moreover, calcium signaling and IL-4 production were defective in Th2 lymphocytes from PKG null mice, supporting a major role for PKG in Th2 cell activation.

We showed that cGMP concentration was increased in stimulated Th2 cells in a similar range as the rise reported in B-cell receptor-activated B lymphocytes (21). Incubation of Th2 lymphocytes with cGMP or SNP induced calcium response and IL-4 mRNA transcripts. This up-regulation of IL-4 expression was due to calcium signaling because it was inhibited by cyclosporin A. However, cGMP or SNP could not induce detectable IL-4 protein. This could be explained by the fact that IL-4 production requires a sustained calcium response in time and amplitude, which may not be induced by cGMP and SNP. Indeed, NFAT nuclear translocation, which reflects the global calcium response, was weaker in cells stimulated by cGMP as compared with TCR-activated Th2 cells. Stimulation of Th2 cells with NONOate, a potent NO donor, induced a stronger calcium response and NFAT translocation than SNP, resulting in detectable IL-4 production. High levels of IL-4 synthesis, as observed in TCR-stimulated Th2 cells, involve other calcium-independent pathways that may not be activated by cGMP or SNP. Therefore, the cGMP pathway is likely necessary but not sufficient to induce optimal Th2 cytokine production.

Some effects of NO result from guanylate cyclase and PKG activation, raising the question of the involvement of NO in Th2 cell activation. NO is produced by many cell types, including dendritic cells, whereas its production in T lymphocytes is unlikely (37). In this report, we showed that cGMP was produced in purified Th2 cells after TCR activation in the absence of antigen-presenting cells, excluding an effect of NO pro-
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Non-excitatory cells, including B lymphocytes (21), NK cells (19), dendritic cells (20), naive T cells (24), Th2 cells (18, 28), and epithelial cells (45, 46). It has been shown that signaling through the B cell receptor was coupled to a guanylate cyclase/cGMP-dependent pathway that controlled DHPR-dependent calcium entry (21). Here we have shown that in Th2 lymphocytes DHPR antagonism by nicardipine reduced cGMP-mediated calcium response, suggesting a role for DHPR in cGMP signaling. Although the structure of DHPR has not been completely elucidated in lymphocytes, DHPR had been related to the α1 calcium channel subunit in B cells (21) as well as in Th2 cells (18) and human T cells (24). In excitable cells, the NO/cGMP/PKG pathway is known to activate or repress DHPR-dependent calcium currents depending upon the cell type (47, 48). How this pathway operates in Th2 lymphocytes will require further investigation.

Our study, therefore, establishes the following sequence of events that lead to cytokine production in Th2 lymphocytes (Fig. 7). TCR stimulation results in guanylate cyclase activation, responsible for cGMP production. cGMP then activates PKG, which may, directly or not, control DHP-sensitive calcium influx. Intracellular calcium increase induces NFAT translocation to the nucleus and subsequent gene expression. Altogether, our data lend support for a new signaling pathway in Th2 lymphocytes in which cGMP acts as a major second messenger in Th2 calcium response and cytokine production.

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FIGURE 7. Schematic representation of the TCR-induced cGMP pathway involved in cytokine production in Th2 lymphocytes. T cell stimulation through the TCR would activate guanylate cyclases, resulting in cGMP level increase. Subsequent PKG activation would be responsible for DHP-sensitive calcium influx, resulting in NFAT translocation to the nucleus and expression of Il4 and Il5 genes. Activators or inhibitors used in this study are indicated.
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