PGE$_2$ Mediated INF-γ Gene Methylation Through cAMP Signaling Pathway in Human Jurkat T Cells

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Received May 18, 2004 / Accepted August 10, 2004

We have examined the effects of S-nitroso-N-acetylpenicillamine (SNAP), prostaglandin E$_2$ (PGE$_2$) and dibutyric cyclic AMP (dbcAMP) on the methylation of interferon-γ (IFN-γ) gene in human Jurkat T cells. The CpG dinucleotide which is critical for promoter function of IFN-γ gene was methylated by treatment with SNAP, PGE$_2$ and dbcAMP, respectively. The DNA methylation induced by PGE$_2$ was suppressed by the addition of 2,5'-dideoxyadenosine (DDA), an inhibitor of adenylly cyclase, but the suppression was not observed in SNAP treated cells. The NO production was not enhanced in PGE$_2$ or dbcAMP treated cells. The methylation induced by PGE$_2$ and dbcAMP was not suppressed by the addition of N$^6$-methyl-L-arginine (L-NMMA), NO synthase inhibitor. In conclusion, the inhibition of IFN-γ gene expression by PGE$_2$ was associated with the methylation of IFN-γ gene by elevation of intracellular cAMP in human Jurkat T cells. However, the methylation induced by PGE$_2$ might not be mediated through the NO production.

Key words – INF-γ, CpG dinucleotide, Methylation, Nitric oxide, PGE$_2$

Methylation of cytosine residues in CpG dinucleotide pairs is an important mechanism through which genes can be differentially transcribed in various cell types. During the past several years, there has been incremental interest in abnormal methylation patterns to affect either activation (hypomethylation) or silencing (hypermethylation) of genes that are important for development, the immune response, and the progression and metastasis of tumors [11,14,19,20]. A striking correlation was observed between the capacity of IFN-γ gene to be expressed and the degree of hy-

Nitric oxide (NO) is a mediator of many different biological responses [24]. NO plays a critical role in immunological responses such as inflammation and autoimmune reactivity [17,24]. NO inhibits the proliferation of Th 1 cells and their production of IL-2 and IFN-γ [25], but that is a potent enhancer on the production of Th 2 type cytokine IL-4 [4]. The expression of inducible nitric oxide synthase (iNOS) mRNA is markedly elevated in the presence of PGE$_2$, and is also increased by elevation of intracellular cAMP [2].

In the promoter region of human INF-γ gene, there is a critical CpG target for methylation at a position (-55) [27]. The deletion of this region results in a significant decrease in promoter activity [5,22]. The methylation of this site has been correlated with the inhibition of IFN-γ gene expression in T-helper cell lines [26]. The restriction endonuclease SmaI (recognition site TACGTA) was found to cut DNA only when the cytosine (C) in the site was not methylated, and would not cut DNA when the C was methylated [27].

Recent year, it was demonstrated that PGE$_2$ inhibited the hypomethylation of the SmaI site of the INF-γ gene in naive CD4$^+$ T cells [12], and the inhibition of INF-γ gene expression by PGE$_2$ is associated with the elevation of
intracellular cAMP and NO in human Jurkat T cells [2]. Hence, it is expected that PGE2 can methylate the SnaBl site of the INF-γ gene by nitric oxide (NO) and cAMP production. We have examined the correlation between silencing of INF-γ gene by PGE2 and the methylation of SnaBl site in human Jurkat T cells.

Materials and Methods

Cell cultures and reagents

The human Jurkat T cell line was maintained in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin (Gibco BRL, Life Technologies, USA) under 5% CO2 at 37°C. Cells were incubated at 1 × 106 cells/ml in culture dishes, and cultures were stimulated with phytohaemagglutinin (PHA, 10 μg/ml, Sigma Chemicals, USA) plus phorbol 12-myristate 13-acetate (PMA, 10 ng/ml, Sigma) on the indicated time points, in the presence or absence of PGE2 (1 μM, Sigma), dbcAMP (10 μM, Sigma) or S-nitroso-N-acetyl-penicillamine (100 μM SNAP, Sigma). For certain experiments, cell cultures were performed with PHA/PMA in the presence of either L-NMMA (500 μM, Sigma) and dbcAMP or PGE2.

PCR analysis of methylation status

Primer pairs designed by Mikovits et al. [20] were used for PCR analysis. Briefly, the primer pairs flanked the methyl-sensitive SnaBl site at -55 of IFN-γ gene promoter. The sense primer of upstream (US) SnaBl (-103~−79) and the antisense (AS) primer (760~−784) yielded a product of 887 bp. As an internal control, the PCR with the downstream (DS) sense primer (319~−343) and the AS primer (760~−784) primer was performed and yielded a product of 466 bp. The product was used as an internal control (Fig. 1) [20]. The 50 ng of DNA were digested with BamHI and SnaBl overnight to ensure complete digestion; 10 ng of DNA was then amplified in a 25 μl PCR mixture. PCR products were electrophoresed on 1.2% agarose gels, and products were visualized by ethidium bromide staining.

Measurement of NO

Nitrite and nitrate, the stable metabolites of NO, were determined in culture medium by the Griess method. NO released from cells in culture medium was quantified by measuring only nitrite using Griess reagent [a combination of equal amount of 0.2% naphthyl ethylenediamine di-

hydrochloride (Sigma) in water and 2% sulfanilamide (Sigma) in 5% H3PO4]. Briefly, 100 μl of supernatant was incubated with an equal volume of Griess reagent at room temperature for 10 min. Then, absorbance at 540 nm was compared with that of NaNO2 (Sigma) standard.

Results

Effects of PGE2, dbcAMP and SNAP on methylation of INF-γ gene in human Jurkat T cells

INF-γ gene expression in human Jurkat T cells was inhibited by treatment of PGE2, dbcAMP and sodium nitroprusside (SNP) [2]. Because methylation of a CpG dinucleotide in the promoter region of INF-γ gene inhibited the gene expression, we have first examined the methylation pattern of the CpG dinucleotide in the presence of PGE2, dbcAMP and SNAP. Human Jurkat T cells were treated with PGE2, dbcAMP and SNAP, respectively, for 24 h, and then, the genomic DNA was extracted and digested with methyl-sensitive enzyme (SnaBl) followed by PCR amplification with the upstream (US)-antisense (AS) primer pair, which spans the SnaBl site. The premise of this method is that DNA cut by SnaBl will not yield a PCR product. The downstream (DS)-AS primer pair, which did not contain a SnaBl site and should have a PCR product, was included as a control for a poor PCR. The method can validate the methylation status of SnaBl site of INF-γ promoter by amplified PCR product by US-AS primer pair (Fig. 1). The methylation of SnaBl site of INF-γ gene was identified by detection of the 887 bp band. As a DNA loading control, PCR was performed with the DS-AS primer pair, which does not flank the SnaBl site and the
product would be independently amplified without regard to the methylation status of SnfBl site. The results showed that identical PCR products (~470 bp) were generated in all samples, the treated cells and the untreated cells.

As shown in Fig. 1, the SnfBl site in the promoter region of IFN-γ gene was methylated by treatment of Jurkat T cells with PGE₂, dbcAMP and SNAP, respectively (Fig. 1, lanes 3-8).

The effects of DDA, adenylyl cyclase inhibitor, on methylation of INF-γ gene induced by PGE₂

Because PGE₂ signaling occurs mainly via cAMP, the methylation of IFN-γ gene induced by PGE₂ may be suppressed by the treatment with DDA, adenylyl cyclase inhibitor. To confirm this prospect, human Jurkat T cells were treated with PGE₂ in the presence of DDA (500 μM). Then, the methylation status of methyl-sensitive SnfBl site was analyzed by PCR amplification. The methylation of SnfBl site induced by PGE₂ was inhibited by treatment with DDA (Fig. 2).

NO production by treatment of PGE₂ and dbcAMP to human Jurkat T cells

Because SNAP induced the methylation of INF-γ gene, we have examined whether the methylation induced by PGE₂ or dbcAMP was associated with NO production. Human Jurkat T cells were incubated for 24 h in the presence of PGE₂ or dbcAMP, then, the culture media were collected and NO concentration was measured using Griess colorimetric assay. Unexpectedly, NO production by SNAP treatment was progressively increased, however, a significant change of NO production by treatment of PGE₂ or dbcAMP was not detected (Fig. 3).

The effects of NO inhibitor (L-NMMA) on methylation of INF-γ gene induced by PGE₂ and dbcAMP

Because a significant elevation of NO production was not detected in Jurkat T cells by treatment with PGE₂ or dbcAMP (Fig. 3), it was not clear whether PGE₂ exert their methylation ability via cAMP-dependent NO production or not. It may be predicted that if PGE₂ or dbcAMP exert INF-γ gene methylation via NO production, NO synthase inhibitor might suppress their methylation ability. To assess whether NO synthase inhibitor suppress the methylation induced by PGE₂ or dbcAMP, human Jurkat T cells were treated with PGE₂ or dbcAMP in the presence of L-NMMA (500 μM). Then, the methylation status of methyl-sensitive SnfBl site was analyzed by PCR amplification. The methylation of SnfBl site induced by dbcAMP and PGE₂ was not inhibited by NO synthase inhibitor (Fig. 4).

Discussion

Expression of cytokines by T lymphocytes is a highly balanced process, involving stimulatory and inhibitory intracellular signaling pathways. Two types of Th cells are distinguished by the pattern of cytokine production. Th 1 cells produce IL-2 and INF-γ, whereas Th 2 cells produce...
IL-4, IL-5 and IL-10. The cytokine profile determines the effector functions of the two subsets of T cells [1]. Th 1 and 2 cell subsets modulate each other’s activity, and the balance between the two subsets determines the outcome of infections and pathophysiological diseases. To regulate the balance in the expression of Th 1 and 2 type cytokine genes is very important in immune response.

DNA methylation plays an important role in the process of gene regulation of specific genes in animal cells. The only known occurrence of DNA methylation in animal cells is in carbon 5 of cytosine, which is localized almost exclusively in CpG residues. Tissue-specific genes generally are fully methylated in almost all cell types of the adult organism. Housekeeping genes contain 5’ CpG islands, which are constitutively unmethylated in all cells [9,10]. The basic mechanism by which DNA methylation affects gene transcription involves altering protein-DNA interactions, especially proteins that are necessary for transcription [8,13,15,16]. Demethylation of the specific sites may render the gene more accessible to the regulatory protein, which initiates the expression of the gene. Katamura et al. [12] demonstrated that IL-4 and PGE2 inhibit hypomethylation of 5’ regulatory region of INF-γ gene during differentiation of naive CD4+ T cells.

Professional antigen-presenting cells, such as macrophages follicular dendrite cells, synthesize PGE2 as major products of arachidonic acid metabolism [13,15]. It has been reported that PGE2 have various effects on many aspects of the immune system, such as immunoglobulin synthesis of B cells and cytokine production by Th cells. PGE2 as well as other reagents that elevate cAMP, inhibits the production of IL-2 and INF-γ by committed Th1-type cells, but does not inhibit IL4 and IL-5 expression [3,6,7,18,21,23]. In many systems, the biological effects of PGE2 are mediated by the increase of cAMP through its specific receptors [27]. The expression of iNOS mRNA was markedly elevated in the presence of PGE2 and NO production by treatment of SNP induced the inhibition of INF-γ gene expression in PHA/PMA-activated human Jurkat T cells [2].

Thus, this study was performed with the expectation that the methylation of INF-γ gene promoter region by PGE2 might be exerted through NO production via cAMP signaling pathway.

The methylation of Snail site of IFN-γ gene was obviously induced by PGE2 and dabcAMP (Fig. 1), the methylation by PGE2 was inhibited by adenyl cyclase inhibitor, DDA (Fig. 2). Thus, PGE2 might mediate IFN-γ gene methylation via cAMP-signaling pathway. Recently, Hmada et al. [8] reported that interleukin (IL)-1β provoked a marked repression of genes, such as fragile X mental retardation 1 (FMR1) and hypoxanthine phosphorotransferase (HPRT), by hypermethylation of CpG island in their promoter region. This effect of IL-1β was mediated through NO produced by induction of NO synthase (iNOS) expression [8]. Since SNAP induced IFN-γ gene methylation, we have examined whether PGE2 and dabcAMP induced the NO production or not. However, we could not detect NO production in culture supernatants by the Griess colorimetric assay (Fig. 3). Benbernou and his colleague detected the iNOS gene expression as an enzyme activity and at the protein level, however, they could not detect NO production by the same Griess colorimetric assay in human Jurkat T cells [2]. So, we suggested that this technique might not be sufficiently sensitive to allow detection of the weak production of NO as in the case of their experimental conditions.

Next, it was examined indirectly whether the elevation of intracellular cAMP concentration are associated with NO production or not. It may be predicted that if these reagents induce the methylation via NO production, NO inhibitor may suppress the methylation of INF-γ gene. To assess the effect of NO inhibitor, human Jurkat T cells were treated with PGE2 and dabcAMP in the presence of 500 μM of L-NMMA as NO inhibitor, and analysed the
methylation status of the SnaBI site by PCR. However, the methylation induced by both reagents, dbcAMP and PGE2, was not inhibited by treatment with 500 μM L-NMMA (Fig. 4, lanes 5, 6 and 9, 10).

In this study, we demonstrated that PGE2, dbcAMP and SNAP induced the methylation of SnaBI site of INF-γ gene, however, PGE2 and dbcAMP did not elevate levels of NO production and NO inhibitor, L-NMMA, did not suppress the methylation induced by PGE2 and dbcAMP. In conclusion, the inhibition of INF-γ gene expression in human Jurkat T cells by treatment of PGE2 could probably be associated with the methylation of INF-γ gene via the elevation of cAMP levels induced by PGE2. However, it would be thought that the methylation might not be concerned with NO production.

Acknowledgments

This study was supported by the grants of Ministry of Health & Welfare, R.O.K., Research and Development of Prescription (Hangbak-tang) for the therapy of Leukemia (#02-PJ3-PGI CO-05-0003).

References


초록: 인간의 Jurkat T세포에서 프로스타글린 E₂ (PGE₂)의 cAMP 경로를 통한 인터페론 감마 (INF-γ) 유전자 methylation

전병훈 ¹· 주성민 ¹· 정재성 ¹· 김영인 ²· 윤용갑 ³· 박 현 ³· 정현택 ²· 한동민· 김현신 ²
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본 연구에서 인간의 백혈병세포주인 Jurkat T 세포에서 인터페론 감마(INF-γ) 유전자의 methylation에 대한 S-nitroso-N-acetylpenicillamine (SNAP)의 프로스타글린 E₂ (PGE₂) 그리고 dibutric cyclic AMP (dbcAMP)에 의한 methylation은 DDA에 의해서 억제되지 않았다. PGE₂나 dbcAMP에 의한 methylation은 아데닐산 사이클로제의 저해제인 2',5'-dideoxycadenosine (DDA)에 의해 억제되었지만, SNAP에 의해서 억제된 methylation은 DDA에 의해서 억제되지 않았다. PGE₂나 dbcAMP를 처리한 세포에서 인산화결소(NO)의 생성의 증가는 나타나지 않았으며, PGE₂나 dbcAMP에 의해 유도된 인터페론 감마유전자 methylation도 인산화결소 생성효소의 저해제인 N⁵-methyl-L-arginine (L-NMMA)에 의해 억제되지 않았다. 따라서 인간의 Jurkat T 세포에서 PGE₂에 의한 인터페론 감마 유전자의 발현 억제는 세포내의 cAMP생성경로를 통한 인터페론 감마 유전자 methylation과 연관되어있으나 인산화결소의 생성경로와는 무관한 것으로 보인다.