Journal of Life Science **2004**, Vol. 14. No. 4. 670~675 ©JLS

PGE_2 Mediated INF-γ Gene Methylation Through cAMP Signaling Pathway in Human Jurkat T Cells

Byung Hun Jeon[1], Sung Min Ju[1], Jae Sung Jeong[1], Myung Wan Kim[1], Young Gap Yun[1], Hyun Park[2], Hun-Taeg Chung[2], Dong Min Han and Won Sin Kim*

Major of Life Science, Division of Natural Science and Technology, College of Natural Sciences and Institute of Basic Natural Sciences, Wonkwang University, Iksan, Chunbuk 570-749, Korea

[1]*College of Oriental Medicine, Wonkwang University, Iksan, Chunbuk 570-749, Korea*

[2]*School of Medicine & Medical Resources Research Center, Wonkwang University, Iksan, Chunbuk 570-749, Korea*

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 dibutric 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 adenylyl 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^G-methyl-L-arginine (L-NMMA), NO synthase inhibitor. In conclusion, the inhibition of INF-γ gene expression by PGE_2 was associated with the methylation of INF-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 hypomethylation. The IFN-γ gene was completely methylated at the CpG dinucleotides of promoter region in thymocytes, neonatal T cells, and adult naive $CD4^+$ T cells that have a low or undetectable capacity to express the IFN-γ gene [19]. PGE_2 as well as other reagents that elevate cAMP inhibits the production of Th 1-type cytokines but does not inhibit Th 2-type cytokines production [13]. It has been demonstrated that cAMP-elevating agents inhibit IL-2 and IFN-γ expression, but don't inhibit IL-4 and IL-5 expression in Th cells [3,15,21].

***Corresponding author**
Tel : +82-63-850-6578, Fax : +82-63-857-8837
E-mail : wsnkim@wonkwang.ac.kr

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 *Sna*BI (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 *Sna*BI 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 PGE_2 can methylate the *Sna*BI 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 PGE_2 and the methylation of *Sna*BI 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% CO_2 at 37℃. Cells were incubated at 1×10^6 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 PGE_2 (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 PGE_2.

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 *Sna*BI site at -55 of IFN-γ gene promoter. The sense primer of upstream (US) *Sna*BI (-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 *Sna*BI 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 dihydrochloride (Sigma) in water and 2% sulfanilamide (Sigma) in 5% H_2PO_4]. 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 $NaNO_2$ (Sigma) standard.

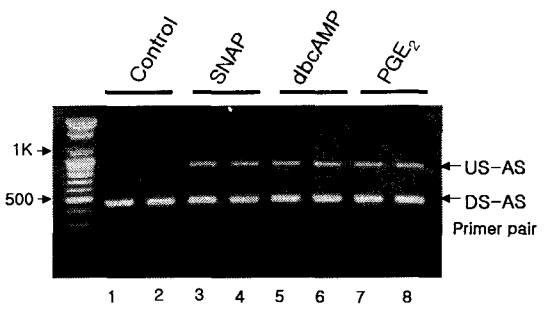

Fig. 1. PCR analysis of the methylation status in INF-γ gene of Human Jurkat T cells. Cells were treated with PGE_2 (1 μM), dbcAMP (10 μM) and SNAP (100 μM) for 24 h, respectively (lanes 3-8). The genomic DNA was digested with *Sna*BI, PCR was performed with the US -AS primer pair and DS-AS primer pair as a DNA loading control.

Results

Effects of PGE_2, 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 PGE_2, 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 PGE_2, dbcAMP and SNAP. Human Jurkat T cells were treated with PGE_2, dbcAMP and SNAP, respectively, for 24 h, and then, the genomic DNA was extracted and digested with methyl-sensitive enzyme (*Sna*BI) followed by PCR amplification with the upstream (US)-antisense (AS) primer pair, which spans the *Sna*BI site. The premise of this method is that DNA cut by *Sna*BI will not yield a PCR product. The downstream (DS)-AS primer pair, which did not contain a *Sna*BI site and should have a PCR product, was included as a control for a poor PCR. The method can validate the methylation status of *Sna*BI site of INF-γ promoter by amplified PCR product by US-AS primer pair (Fig. 1). The methylation of *Sna*BI 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 *Sna*BI site and the

product would be independently amplified without regard to the methylation status of *Sna*BI 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 *Sna*BI site in the promoter region of IFN-γ gene was methylated by treatment of Jurkart T cells with PGE_2, dbcAMP and SNAP, respectively (Fig. 1, lanes 3-8).

The effects of DDA, adenylyl cyclase inhibitor, on methylation of INF-γ gene induced by PGE_2

Because PGE_2 signaling occurs mainly via cAMP, the methylation of IFN-γ gene induced by PGE_2 may be suppressed by the treatment with DDA, adenylyl cyclase inhibitor. To confirm this prospect, human Jurkat T cells were treated with PGE_2 in the presence of DDA (500 μM). Then, the methylation status of methyl-sensitive *Sna*BI site was analyzed by PCR amplification. The methylation of *Sna*BI site induced by PGE_2 was inhibited by treatment with DDA (Fig. 2).

NO production by treatment of PGE_2 and dbcAMP to human Jurkat T cells

Because SNAP induced the methylation of INF-γ gene, we have examined whether the methylation induced by PGE_2 or dbcAMP was associated with NO production. Human Jurkat T cells were incubated for 24 h in the presence of PGE_2 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 PGE_2 or dbcAMP was not detected (Fig. 3).

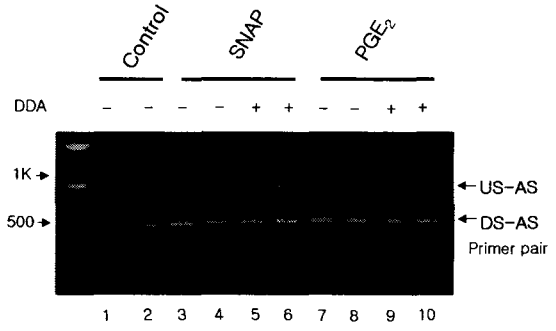

Fig. 2. The effects of DDA (2',5'-dideoxyadenosine) on the methylation of INF-γ gene induced by SNAP or PGE_2. Cells were treated with SNAP (100 μM, lanes 3-6) and PGE_2 (1 μM, lanes 7-10) for 24 h in the presence or absence of 500 μM cAMP inhibitor, 2',5'-dideoxyadenosine. The genomic DNA was digested with *Sna*BI, PCR was performed with the US -AS primer pair and DS-AS primer pair as a DNA loading control.

The effects of NO inhibitor (L-NMMA) on methylation of INF-γ gene induced by PGE_2 and dbcAMP

Because a significant elevation of NO production was not detected in Jurkat T cells by treatment with PGE_2 or dbcAMP (Fig. 3), it was not clear whether PGE_2 exert their methylation ability via cAMP-dependent NO production or not. It may be predicted that if PGE_2 or dbcAMP exert INF-γ gene methylation via NO production, NO synthase inhibitor might be suppress their methylation ability. To assess whether NO synthase inhibitor suppress the methylation induced by PGE_2 or dbcAMP, human Jurkat T cells were treated with PGE_2 or dbcAMP in the presence of L-NMMA (500 μM). Then, the methylation status of methyl-sensitive *Sna*BI site was analyzed by PCR amplification. The methylation of *Sna*BI site induced by dbcAMP and PGE_2, 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

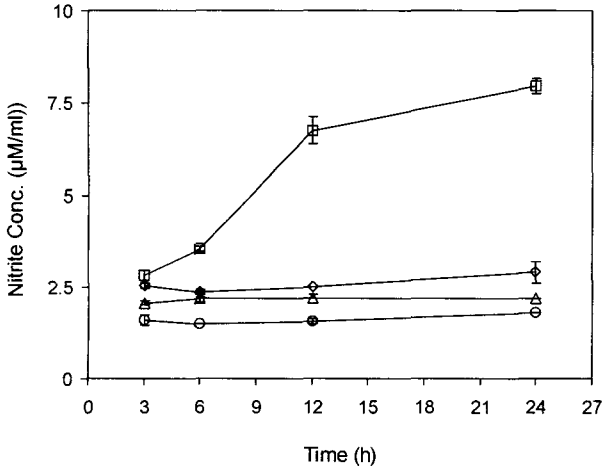

Fig. 3. Time course of NO production in human Jurkat T cells by treatment with PGE_2, dbcAMP and SNAP, respectively. Control (○), 100 μM SNAP (□), 10 μM dbcAMP (△) and 1 μM PGE_2 (◇). NO synthesis was measured by the Griess colorimetric assay. Values are means ± S.D. of three experiments.

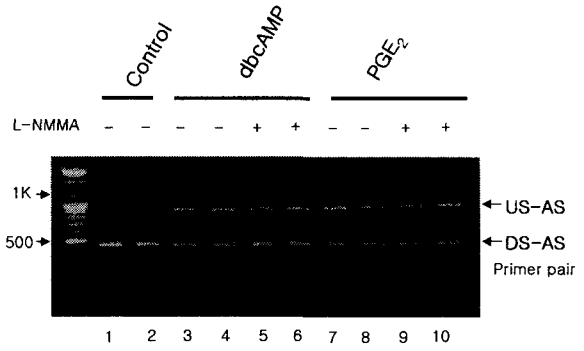

Fig. 4. The effects of L-NMMA on the methylation of INF-γ gene induced by PGE_2 or dbcAMP. Cells were treated with dbcAMP (10 μM, lanes 3-6) and PGE_2 (1 μM, lanes 7-10) for 24 h in the presence or absence of 500 μM NO inhibitor, L-NMMA. The genomic DNA was digested with *Sna*BI, PCR was performed with the US -AS primer pair and DS-AS primer pair as a DNA loading control.

IL-4, IL-5 and IL-10. The cytokine profile determines the effecter 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 PGE_2 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 PGE_2 as major products of arachidonic acid metabolism [13,15]. It has been reported that PGE_2 have various effects on many aspects of the immune system, such as immunoglobulin synthesis of B cells and cytokine production by Th cells. PGE_2, 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 *IL*4 and *IL*-5 expression [3,6, 7,18,21,23]. In many systems, the biological effects of PGE_2 are mediated by the increase of cAMP through its specific receptors [27]. The expression of iNOS mRNA was markedly elevated in the presence of PGE_2 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 PGE_2 might be exerted through NO production via cAMP signaling pathway.

The metylation of *Sna*BI site of IFN-γ gene was obviously induced by PGE_2 and dbcAMP (Fig. 1), the methylation by PGE_2 was inhibited by adenylyl cyclase inhibitor, DDA (Fig. 2). Thus, PGE_2 might mediate IFN-γ gene metylation via cAMP-signaling pathway. Recently, Hmadcha *et al.* [8] reported that interleukin (IL)-1β provoked a marked repression of genes, such as fragile X mental retardation 1 (FMR1) and hypoxantine phosphotransferase (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 metylation, we have examined whether PGE2 and dbcAMP 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 Jurkart 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 PGE_2 and dbcAMP in the presence of 500 μM of L-NMMA as NO inhibitor, and analysed the

methylation status of the *Sna*BI 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 *Sna*BI site of INF-γ gene, however, PGE_2 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 PGE_2 could probably be associated with the methylation of INF-γ gene via the elevation of cAMP levels induced by PGE_2. 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-PJ9-PGI CO-05-0003).

References

1. Becker, P. B., S. Ruppert, and G. Schutz. 1987. Genomic footprinting reveals cell type-specific DNA binding of ubiquitous factors. *Cell* **51**, 435-443.
2. Benbernou, N., S. Esnault, H. C. Shin, H. Fekkar and M. Guenounou. 1997. Differential regulation of IFN-gamma, IL-10 and inducible nitric oxide synthase in human T cells by cyclic AMP-dependent signal transduction pathway. *Immunology* **91**, 361-368.
3. Betz, M. and B. S. Fox. 1991. Prostaglandin E_2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J. Immunol.* **146**, 108-113.
4. Chang, R. H., M. H. Feng, W. H. Liu and M. Z. Lai. 1997. Nitric oxide increased interleukin-4 expression in T lymphocytes. *Immunology* **90**, 364-369.
5. Chrivia, J. C., T. Wedrychowicz, H. A. Young and K. J. Hardy. 1990. A model of human cytokine regulation based on transfection of interferon gamma gene fragments directly into isolated peripheral blood T lymphocytes. *J. Exp. Med.* **172**, 661-664.
6. Gajewski, T. F., S. R. Schell and F. W. Fitch. 1990. Evidence implicating utilization of different T cell receptor-associated signaling pathways by TH1 and TH2 clones. *J. Immunol.* **144**, 4110-4120.
7. Gray, J.G., G. Chandra, W. C. Clay, S. W. Stinnett, S. A. Haneline, J. J. Lorenz, I. R. Patel, G. B. Wisely, P. J. Furdon and J. D. Taylor. 1993. A CRE/ATF-like site in the upstream regulatory sequence of the human interleukin 1 beta gene is necessary for induction in U937 and THP-1 monocytic cell lines. *Mol. Cell. Biol.* **13**, 6678-6689.
8. Hmadcha, A., F. J. Bedoya, F. Sobrino and E. Pintado. 1999. Methylation-dependent gene silencing induced by interleukin 1beta via nitric oxide production. *J. Exp. Med.* **190**, 1595-1604.
9. Issa, J. P., P. M. Vertino, J. Wu, S. Sazawal, P. Celano, B. D. Nelkin, S. R. Hamilton and S. B. Baylin. 1993. Increased cytosine DNA-methyltransferase activity during colon cancer progression. *J. Natl. Cancer. Inst.* **85**, 1235-1240.
10. Johnson, G. S., R. M. Friedman and I. Pastan. 1971. Restoration of several morphological characteristics of normal fibroblasts in sarcoma cells treated with adenosine-3': 5'-cyclic monphosphate and its derivatives. *Proc. Natl. Acad. Sci. USA.* **68**, 425-429.
11. Jones, P. A. 1996. DNA methylation errors and cancer. *Cancer. Res.* **56**, 2463-2467.
12. Katamura, K., T. Fukui, T. Kiyomasu, J. Iio, G. Tai, H. Ueno, T. Heike, M. Mayumi and K. Furusho. 1998. IL-4 and prostaglandin E_2 inhibit hypomethylation of the 5' regulatory region of IFN-gamma gene during differentiation of naive $CD4^+$ T cells. *Mol. Immunol.* **35**, 39-45.
13. Katamura, K., N. Shintaku, Y. Yamauchi, T. Fukui, Y. Ohshima, M. Mayumi and K. Furusho. 1995. Prostaglandin E_2 at priming of naive $CD4^+$ T cells inhibits acquisition of ability to produce IFN-gamma and IL-2, but not IL-4 and IL-5. *J. Immunol.* **155**, 4604-4612.
14. Laird, P. W. and R. Jaenisch. 1994. DNA methylation and cancer. *Hum. Mol. Genet.* **3**, 1487-1495.
15. Lee, H. J., N. Koyano-Nakagawa, Y. Naito, J. Nishida, N. Arai, K. Arai and T. Yokota. 1993. cAMP activates the IL-5 promoter synergistically with phorbol ester through the signaling pathway involving protein kinase A in mouse thymoma line EL-4. *J. Immunol.* **151**, 6135-6142.
16. Leonhardt, H. and T. H. Bestor. 1993. Structure, function and regulation of mammalian DNA methyltransferase. In DNA methylation: molecular biology and biological significance (Jost JP, Saluz HP, eds), 109-119, Birkhauser Verlag, Basel, Switzerland.
17. Liew, F. Y. 1995. Regulation of lymphocyte functions by nitric oxide. *Curr. Opin. Immunol.* **7**, 396-399.
18. Mary, D., C. Aussel, B. Ferrua and M. Fehlmann. 1987. Regulation of interleukin 2 synthesis by cAMP in human T cells. *J. Immunol.* **139**, 1179-1184.
19. Melvin, A. J., M. E. McGurn, S. J. Bort, C. Gibson and D. B. Lewis. 1995. Hypomethylation of the interferon-gamma gene correlates with its expression by primary T-lineage cells. *Eur. J.Immunol.* **25**, 426-430.
20. Mikovits, J. A., H. A. Young, P. Vertino, J-P. Issa, P. M. Pitha, S. Turcoski-Corrales, D. D. Taub, C. L. Petrow, S. B. Baylin and F. W. Ruscetti. 1998. Infection with Human Immunodeficiency Virus Type 1Upregulates DNA Methyltransferase, Resulting in De Novo Methylation of the Gamma Interferon (IFN-γ) Promoter and Subsequent Downregulation of IFN-γ Production. *Mol. Cell. Biol.* **18**,

5166-5177.
21. Munoz, E., A. M. Zubiaga, M. Merrow, N. P. Sauter and B. T. Huber. 1990. Cholera toxin discriminates between T helper 1 and 2 cells in T cell receptor-mediated activation: role of cAMP in T cell proliferation. *J. Exp. Med.* **172**, 95-103.
22. Penix, L., W. M. Weaver, Y. Pang, H. A. Young and C. B. Wilson. 1993. Two essential regulatory elements in the human interferon gamma promoter confer activation specific expression in T cells. *J. Exp. Med.* **178**, 1483-1496.
23. Snijdewint, F. G., P. Kalinski, E. A. Wierenga, J. D. Bos and M. L. Kapsenberg. 1993. Prostaglandin E_2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J. Immunol.* **150**, 5321-5329.
24. Schmidt, H. H. and U. Walter. 1994. NO at work. *Cell.* **78**, 919-925.
25. Taylor-Robinson, A. W., F. Y. Liew, A. Severn, D. Xu, S. J. McSorley, P. Garside, J. Padron and R. S. Phillips. 1994. Regulation of the immune response by nitric oxide differentially produced by T helper type 1 and T helper type 2 cells. *Eur. J. Immunol.* **24**, 980-984.
26. Young, H. A., P. Ghosh, J. Ye, J. Lederer, A. Lichtman, J. Gerard, L. Penix, C. B. Wilson, A. J. Melvin, M. E. McGurn, D. B. Lewis and D. D. Taub. 1994. Differentiation of the T helper phenotypes by analysis of the methylation state of the IFN-γ gene in immune cell regulation. *J. Immunol.* **153**, 3603-3610.
27. Young, H. A. 1996. Regulation of interferon-gamma gene expression. *J. Interferon. Cytokine. Res.* **16**, 563-568.

초록 : 인간의 Jurkat T세포에서 프로스타글란딘 E_2 (PGE_2)의 cAMP 경로를 통한 인터페론 감마 (INF-γ) 유전자의 methylation

전병훈[1] · 주성민[1] · 정재성[1] · 김명완[1] · 윤용갑[1] · 박 현[2] · 정헌택[2] · 한동민 · 김원신*
(원광대 자연대학 생명과학부, [1]원광대학교 한의과대학, [2]원광대학교 의과대학)

본 연구에서 인간의 백혈병세포주인 Jurkat T 세포에서 인터페론 감마(INF-γ) 유전자의 methylation에 대한 S-nitroso-N-acetylpenicillamine (SNAP), 프로스타글란딘 E_2 (PGE_2) 그리고 dibutric cyclic AMP (dbcAMP)의 효과를 조사하였다. 인터페론 감마 유전자의 프로모터기능에 아주 중요한 디뉴클레오티드인 CpG는 SNAP, PGE_2, 그리고 dbcAMP를 각각 처리하였을 때 methylation되었다. PGE_2에 의해서 유도된 그 methylation은 아데닐산 사이클라제의 저해제의 하나인 2',5'-dideoxyadenosine (DDA)에 의해서 억제되었지만, SNAP에 의해서 유도된 methylation은 DDA에 의해서 억제되지 않았다. PGE_2나 dbcAMP를 처리한 세포에서 일산화질소(NO)의 생성의 증가는 나타나지 않았으며, PGE_2나 dbcAMP에 의해 유도된 인터페론 감마유전자의 methylation도 일산화질소합성효소의 저해제인 N^G-methyl-L-arginine (L-NMMA)에 의해서 억제되지 않았다. 따라서 인간의 Jurkat T 세포에서 PGE_2에 의한 인터페론 감마 유전자의 발현 억제는 세포내의 cAMP생성경로를 통한 인터페론 감마 유전자의 methylation과 연관되어있으나 일산화질소의 생성경로와는 무관한 것으로 보인다.