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Effect of Trichostatin A on CD4 surface density in peripheral blood T cells

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Abstract: Acetylation level of chromatin histones is maintained by histone acetylases (HATs) and deacetylases (HDACs) and correlates with transcriptional activity of genes. Trichostatin A (TSA) is HDAC inhibitor that causes various effects in cells, including immunomodulation. The CD4 antigen is a key coreceptor involved in activation of T helper cells. Using quantitative real-time PCR (RQ-PCR) and flow cytometry techniques, we estimated CD4 transcript level and density of CD4 antigen on the surface of TSA-treated stimulated and unstimulated peripheral T cells. We observed a dose dependent decrease in CD4 mRNA level and antigen density on surface of TSA-treated stimulated T cells. However, we did not observe any significant TSA effect on CD4 mRNA and protein expression in unstimulated T cells. Our data suggest that TSA may induce biosynthesis of factors responsible for negative regulation of CD4 antigen expression in stimulated T cells. Our investigation may support previous observation that this drug has immunosuppresive effect on primary T cells and may be useful in treatment of certain autoimmune disorders.

Key words: Trichostatin A - Histone deacetylases - CD4 antigen - Flow cytometry

Introduction

Chromatin structure is controlled by the level of core histone acetylation, which is determined by the balance between histone acetyl transferase (HAT) and histone deacetylase (HDAC) activity [19]. Changes in chromatin acetylation are a key event in transcriptional regulation of genes. Hyperacetylation of histones is associated with transcriptional activity, whereas histone hypoacetylation represses gene expression [7, 19]. Thus, regulation of gene expression includes transcription factors, which recruit HATs and HDACs to acetylate and deacetylate histones and modify chromatin structure [7, 19].

CD4 is a cell surface antigen, its presence is essential for T cell stimulation and function [8]. The T cells stimulation is triggered by direct interaction of the T cell receptor /CD3 complex (CD3/TCR) with an antigen bound to the major histocompatibility complex (MHC) on antigen presenting cells (APC) such as dendritic cells, B lymphocytes and macrophages [5, 8, 10, 11, 13]. During $CD4^+$ T cells stimulation, CD4 binds to a monomorphic region of the MHC class II molecule and increases the avidity of T cell for APC. Interaction of the CD4 with the MHC II molecule enables lck tyrosine kinase to phosphorylate the CD3zeta chain of the CD3/TCR complex. Phosphorylation of CD3zeta begins cascade of events leading to lymphocyte activation and biosynthesis of cytokines and costimulatory/adhesion molecules that can initiate humoral or cytotoxic response [2]. $CD4^+$ T lymphocytes are considered to play a key role in loss of self-tolerance and CD4 antigen can be associated with the development of certain autoimmune diseases [4, 15]. Modulation of CD4 expression may decrease activation threshold and can be the target for immunotherapy [2].

Trichostatin A (TSA) is a reversible HDAC inhibitor that deregulates gene expression and affects cell proliferation and differentiation [14, 16]. High concentrations of TSA cause apoptosis of various cell types [12, 14, 16, 21]. It has been recently reported that TSA at lower concentrations may act as an immunomodulator, altering expression of some genes encoding proteins involved in immune response, such as cytokines, costimulatory/adhesion molecules and MHC class II antigens [14, 16]. Thus, this drug might be useful in the treatment of certain autoimmune disorders.

We previously reported that TSA significantly lowered transcription level of Elf-1 in Jurkat T leukemia cells [13]. Elf-1 is a member of the E-26 specific (Ets)

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family transcription factors that is also implicated in the transcriptional regulation of genes such as CD3-ζ, IL-2, IL-2R, α-chain, granulocyte-macrophage colony-stimulating factor (GM-CSF), blk, lck, and lyn kinases [18, 20]. Elf-1 binds to the CD4 promoter and is critical for its function [18, 20].

Employing quantitative real-time PCR (RQ-PCR) and flow cytometry we evaluated the effect of TSA on CD4 mRNA level and surface density in human stimulated and unstimulated peripheral T lymphocytes.

Materials and methods

Reagents and cell culture. The peripheral blood samples were collected from volunteers and were used to obtain peripheral blood mononuclear cells (PBMCs). The PMBCs were isolated by centrifugation over Ficoll-Hypaque (d=1.077 g/cm^3) [6]. Phytohaemagglutinin (PHA) treatment, in contrast to CD28/CD3 crosslinking, stimulates naive T cells to produce a wide range of cytokines that depend on expression of AP1 and NFkappaB transcription factors [17]. Additionally, in PHA stimulated T cells no significant decrease in cell surface CD4 density is observed [1], and after 4 h stimulation most cells exhibit CD69 expression and high rate of proliferation. Freshly isolated PBMCs were incubated for 4 h either without or in the presence of 10 μg/ml PHA. The cells were then washed and cultured for additional 72 h in the RPMI 1640 medium (GibcoBRL, Grand Island, NY, USA) in a mixture of 5% CO_2 and 95% air at 37°C. The culture medium was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, penicillin/streptomycin mix (Sigma Chemical, St Louis, MO, USA) and recombinant interleukin-2 (rIL-2, 40 U/ml, R&D Systems Inc, Minneapolis, MI, USA). Stimulated and unstimulated PBMCs were suspended at a density of 0.5×10^6 cells/ml in RPMI medium and 1 ml cell suspensions were placed in 24-well polystyrene plates. Plates were then incubated for 24 h either without or in the presence of TSA at concentrations of 3, 30 and 300 nM [9].

RQ-PCR analysis of CD4 transcript level in PBMCs. Total RNA was isolated from PBMCs according to Chomczyński and Sacchi method (1985) [3]. RNA integrity was confirmed by denaturing agarose gel electrophoresis, and the concentration was quantified by measuring the optical density (OD) at 260 nm. RNA samples were treated with DNase I, and reverse-transcribed into cDNA using oligo-dT primers. RQ-PCR was conducted in a Light Cycler real-time PCR detection system (Roche Diagnostics GmbH, Mannheim, Germany) using SYBR® Green I as detection dye and target cDNA was quantified using relative quantification method. The quantity of CD4 transcript in each sample was standardized by polymerase II cDNA levels. The CD4 cDNA amplicon 205 bp was amplified employing pair of primers: (5' CTGGCTCTGGAAACCTCAC 3') (forward, nt 1026-1045) and (5' ACCCACACCGCCTTCTCC 3') (reverse, nt 1213-1231). Polymerase II cDNA 163 bp amplicon was also amplified using primer pairs: (5' GCAAATTCACCAAGAGAGAC 3') (forward, nt 3615-3635) and (5 ATGTGACCAGGTATGATGAG 3') (reverse, nt 3758-3778). The CD4 and polymerase II cDNA primers were respectively designed based on sequences ENST00000011653 and ENST00000322644 located in Ensembl Genome Browser (www.ensembl.org). For amplification, 2 μl of cDNA was added to 18 μl of PCR mix containing HotStartTaq DNA polymerase, reaction buffer, dNTP mix, SYBR Green I dye, 2.5 mM $MgCl_2$ (Roche Diagnostics GmbH, Mannheim, Germany) and primers. One RNA sample of each preparation was processed without RT-reaction to provide a negative control in subsequent PCR reactions. RQ-PCR results are expressed as a percentage of their respective controls (Fig. 1).

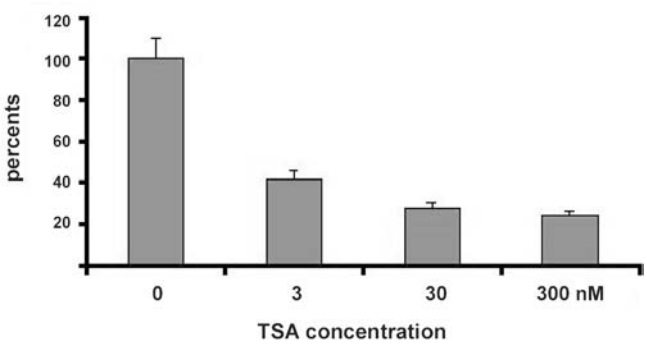

Fig. 1. TSA down-regulates CD4 mRNA in stimulated PBMCs. Stimulated PBMCs were incubated either without or in the presence of TSA concentration of 3, 30 and 300 nM. RQ-PCR analysis results are expressed as a percentage of their respective controls. The results were standardized by Polymerase II transcript level. Each sample was determined in triplicate and results represent means ± SE from three experiments

Flow cytometry. For direct flow cytometric analysis of the CD4 density on the T cell surface, 1×10^5 cells were harvested and stained with Tritest™ (Becton-Dickinson, San Jose, CA, USA) Fluorescein isothiocyanate (FITC)/ Peridinin chlorophyll protein (PerCP) conjugated with anti-CD4/CD3 monoclonal antibodies, or negative isotype controls. The cells were immediately analyzed on FACSCanto Flow Cytometer (Becton-Dickinson, San Jose, CA, USA). The decrease in CD4 molecule density on cell surface was calculated according to 100-(MFx-MFo)/(MFc-MFo) × 100, where MF is the mean fluorescence intensity of cell stained with FITC-conjugated anti CD4, in the presence of (MFx) or absence (MFc) of TSA. Control represents fluorescence of cell stained with an appropriate isotope antibody (MFo).

Results and discussion

There is an increasing evidence that TSA has immunomodulatory potential [14, 16]. TSA represses expression of costimulatory and adhesion molecules including CD11c, CD69, CD28, CD40 and CD40L [16]. However, TSA may also up-regulate expression of CD2, CD6, CD82 antigens as well as transforming growth factor β1 (TGFβ1), CX3C chemokine receptor type 1 and other proteins involved in immune response [16].

It has been recently reported that TSA reduces stability and therefore mRNA level of several genes [19]. Our investigation has revealed that TSA causes a dose-dependent decrease in CD4 mRNA in stimulated PBMCs. Reduction in transcript level achieved 58%, 72% and 76% of their respective controls at TSA concentration of 3, 30 and 300 nM, respectively (Fig. 1). At the concentrations 3 and 30 nM, no apoptotic death was observed (data not shown). This confirms that CD4 mRNA decrease is due to HDAC inhibition, rather than to cytotoxic effect of TSA. However, TSA did not change mRNA level in unstimulated T cells (results not shown).

In order to examine whether low mRNA level leads to decrease in CD4 protein, we determined CD4 den-

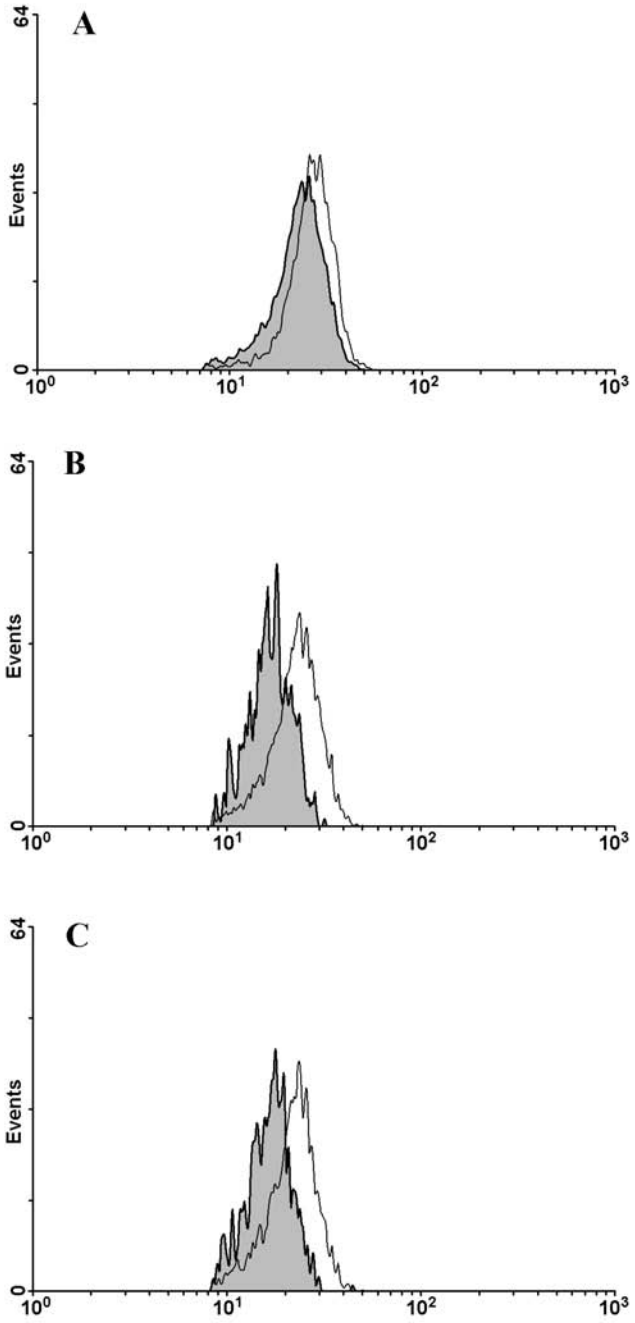

Fig. 2. Representative picture of flow cytometric analysis of CD4 surface density in stimulated T cells incubated in the presence of 3 nM (**A**), 30 nM (**B**) and 300 nM (**C**) TSA. Shadowed and unshadowed graphs represent expression of CD4 in cells incubated with or without TSA, respectively.

sity on cell surface. We observed a dose dependent decrease in CD4 density on surface of stimulated T cells. The decrease in CD4 surface density achieved 8%, 40% and 41% in TSA concentration of 3, 30 and 300 nM, respectively (Fig. 2). However, we did not observe any significant TSA effect on CD4 density on surface of unstimulated T cells (data not shown).

We previously reported that TSA significantly lowered level of transcription of Elf-1 in Jurkat T leukemia cells [13]. Negative effect of TSA on Elf-1 expression may partially explain decrease in CD4 mRNA and density of CD4 protein on surface of T cells.

The differences between effects of TSA on CD4 density on cell surface may result from different histone acetylation level in stimulated and unstimulated T cells [7, 19]. Stimulated T cells contain high number of acetylated histones in chromatin structure that makes DNA template available for transcription complex [7, 19]. However, the final effect of chromatin hyperacetylation on transcription of particular gene has to be determined experimentally.

TSA is now being considered to be promising anticancer drug [16, 21]. Our findings suggest that TSA at low concentration may also have implications in therapy of autoimmune disease, in which exaggerated CD4 mediated response is observed. The further detailed investigation of TSA effect on expression of other genes coding proteins involved in immune response may provide valuable explanation of mechanism of T cell suppression.

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References

[1] Baran J, Kowalczyk D, Ozog M, Zembala M (2001) Three-color flow cytometry detection of intracellular cytokines in peripheral blood mononuclear cells: comparative analysis of phorbol myristate acetate-ionomycin and phytohemagglutinin stimulation. Clin Diagn Lab Immunol 8: 303-313
[2] Bryl E, Gazda M, Foerster J, Witkowski JM (2001) Age-related increase of frequency of a new, phenotypically distinct subpopulation of human peripheral blood T cells expressing lowered levels of CD4. Blood 98: 1100-1107
[3] Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156-159
[4] Fiori P, Ristori G, Cacciani A, Buttinelli C, Falcone M, Giovanni SD, Montesperelli C, Pozzilli C, Salvetti M (1997) Down-regulation of cell-surface CD4 co-receptor expression and modulation of experimental allergic encephalomyelitis. Int Immunol 9: 541-545
[5] Fowlkes, BJ, Pardoll D (1989) Molecular and cellular events of T cell development. Adv Immunol 44: 207-264
[6] Hoser G, Wasilewska D, Domagała-Kulawik J (2004) Expression of Fas receptor on peripheral blood lymphocytes from patients with non-small cell lung cancer. Folia Histochem Cytobiol 42: 249-252
[7] Huo X, Zhang J (2005) Important roles of reversible acetylation in the function of hematopoietic transcription factors. Cell Mol Med 9: 103-112
[8] Jakóbisiak M (2004) Immunologia (in Polish). PWN, Warszawa
[9] Januchowski R, Jagodzinski PP (2005) Effect of 5-azacytidine and procainamide on CD3-zeta chain expression in Jurkat T cells. Biomed Pharmacother 59: 122-126

[10] Januchowski R, Jagodzinski PP (2005) Effect of procainamide on transcription of ZAP-70, Syk, LAT, and SLP-76 signal molecules genes in Jurkat T cells. Pol J Environ Studies 14, Suppl 2: 156-159
[11] Jóźwik A, Soroczyńska M, Witkowski JM, Bryl E (2004) CD3 receptor modulation in Jurkat leukemic cell line. Folia Histochem Cytobiol 42: 41-43
[12] Karagiannis TC, Harikrishnan KN, El-Osta A (2005) The histone deacetylase inhibitor, Trichostatin A, enhances radiation sensitivity and accumulation of gammaH2A.X. Cancer Biol Ther 4: 787-793
[13] Kozłowska A, Jagodziński PP (2005) Effect of procainamide, hydralazine and trichostatin A on Elf-1 transcription level in Jurkat T cells. Pol J Environ Studies 14, Suppl 2: 243-246
[14] Koyama Y, Adachi M, Sekiya, Takekawa M, Imai K (2000) Histone deacetylase inhibitors suppress IL-2-mediated gene expression prior to induction of apoptosis. Blood 96: 1490-1495
[15] Krieger NR, Fathman CG, Shaw MK, Ridgway WM (2000) Identification and characterization of the antigen-specific subpopulation of alloreactive CD4+ T cells *in vitro* and *in vivo*. Transplantation 69: 605-609
[16] Moreira JMA, Scheipers P, Sorensen P (2003) The histone deacetylase inhibitor Trichostatin A modulates CD4+ T cell responses. BMC Cancer 3: 30
[17] Roeder M, Raju PA, Dipendra KM, Herzenberg LA, Herzenberg LA (1997) HIV does not replicate in naive CD4T cells stimulated with CD3/CD28. J Clin Invest 99: 1555-1564
[18] Sarafova S, Siu G (1999) A potential role for Elf-1 in CD4 promoter function. J Biol Chem 274: 16126-16134
[19] Sengupta N, Seto E (2004) Regulation of histone deacetylase activities. J Cell Biochem 93: 57-67
[20] Wurster AL, Siu G, Leiden JM, Hedrick SM (1994) Elf-1 binds to a critical element in a second CD4 enhancer. Mol Cell Biol 14: 6452-6463
[21] Xiong Y, Dowdy SC, Podratz KC, Jin F, Attewell JR, Eberhardt NL, Jiang SW (2005) Histone deacetylase inhibitors decrease DNA methyltransferase-3B messenger RNA stability and down-regulate de novo DNA methyltransferase activity in human endometrial cells. Cancer Res 65: 2684-2689

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