Cellular Immunology 287 (2014) 78–85

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Requirement of the expression of 3-phosphoglycerate dehydrogenase for traversing S phase in murine T lymphocytes following immobilized anti-CD3 activation

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ARTICLE INFO

Article history:
Received 11 February 2008
Accepted 5 December 2013
Available online 18 December 2013

Keywords:
L-Serine biosynthesis
The phosphorylated pathway
PHGDH
T cell activation
IL-2R signaling
S phase
Antisense oligonucleotide

ABSTRACT

Murine resting (G_0) T lymphocytes contained no detectable mRNA of 3-phosphoglycerate dehydrogenase (PHGDH) catalyzing the first step in the phosphorylated pathway of L-serine biosynthesis. Immobilized anti-CD3 activation of G_0 T cells expressed the PHGDH mRNA in G_1 with a maximum level in S phase. G_0 T cells activated with either immobilized anti-CD3 plus CsA or PBu_2, which failed to drive the activated T cells to enter S phase, did not express the PHGDH mRNA unless exogenous rIL-2 was added. Blocking of IL-2R signaling by adding anti-IL-2 and anti-IL-2Rα resulted in no expression of the PHGDH mRNA during immobilized anti-CD3 activation of G_0 T cells. Deprivation of L-serine from culture medium or addition of antisense PHGDH oligonucleotide significantly reduced [^{3}H]TdR incorporation of activated T cells. These results indicate that the PHGDH gene expression, dictated by IL-2R signaling, is a crucial event for DNA synthesis during S phase of activated T cells.

1. Introduction

L-Serine is required not only for protein synthesis but also for various metabolic pathways to generate several essential compounds including glycine, cysteine, D-serine, phosphatidylserine, sphingomyelins, and cerebrosides [1]. It is also utilized for synthesis of nucleotide precursors, which is linked to cellular replication, and for redox control, which can contribute to cell survival [2]. Although L-serine is available from dietary sources, it can be endogenously synthesized from glycolytic intermediate by the phosphorylated pathway in mammals. The phosphorylated pathway starts at 3-phosphoglycerate and sequentially proceeds three stages of enzymatic reactions to synthesize L-serine. The 3-phosphoglycerate dehydrogenase (PHGDH) catalyses the transition of 3-phosphoglycerate into 3-phosphohydroxy pyruvate, which is the first and rate-limiting step in the phosphorylated pathway, using NAD^+/NADH as a cofactor. Phosphoserine aminotransferase (PSAT) and phosphoserine phosphatase (PSP) catalyze the conversion of 3-phosphohydroxy pyruvate to 3-phosphoserine and subsequent dephosphorylation of 3-phosphoserine to L-serine, respectively.

It has been reported that the enzyme activity of PHGDH is elevated eight- to 50-fold in rat hepatoma [3], 10-fold in human colon carcinoma, and 32-fold in rat sarcoma [4] as compared with individual normal control values. It has also been shown that PHGDH expression at the transcription level is upregulated in human colon carcinoma, and in most leukemias and lymphomas of human and murine origin [5]. This elevated activity of PHGDH, which leads to the enhanced capacity of L-serine biosynthesis, has been interpreted as an acquired growth advantage of tumor cells because of the metabolic importance of L-serine in nucleotide biosynthesis [3,4]. In healthy humans, de novo synthesis of L-serine by the phosphorylate pathway is essential to supply the L-serine required in the brain, because the delivery of L-serine to the central nervous system (CNS) is insufficient due to the blood–brain barrier [6,7]. In relation to the role of L-serine in the CNS, L-serine and its downstream metabolite glycine serve as the neurotrophic factor to promote survival, dendritogenesis, and electrophysiological development of neurons [8]. In addition, D-serine, which acts as a co-agonist of the N-methyl-D-aspartate (NMDA) receptor along with glutamate and glycine, is directly formed from L-serine by serine racemase [9,10]. The importance of L-serine synthesis via the phosphorylated pathway in the brain has also been verified by human inborn disorder with enzymic defects in PHGDH, which results in a severe neurological syndrome [11], and by the PHGDH-knockout mice model exhibiting early embryonic lethality associated with multiple neurodevelopmental defects [12,13].

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0008-8749/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cellimm.2013.12.003

The importance of L-serine in cellular replication and thus highest capacity of the phosphorylated pathway in S phase of the cell cycle are supported by our previous results, which showed that the level of mRNA specific for both PHGDH and PSAT, abruptly down-regulated in accordance with growth arrest of U937 cells on 12-O-tetradecanoylphorbol 13-acete (TPA)-induced monocytic differentiation, can be recovered when TPA-treated cells restore cell growth by a retrodifferentiation process, and that the level of PHGDH- and PSAT-specific mRNA fluctuates during the cell cycle with a maximum in S phase of human Jurkat T cells [5,14]. It has also been shown that ectopic overexpression of PSAT in colon cancer cell line results in an enhancement in its growth rate and survival [15]. Although these previous results suggested that the expression of PHGDH gene, which contributes to the phosphorylated pathway, might be a prerequisite for traversing S phase, there has been no direct evidence showing the requirement of PHGDH gene expression for S phase during cell cycle progression.

In the present study, the expression of PHGDH gene was investigated during immobilized anti-CD3 activation of murine splenic resting (G_0) T lymphocytes, with focusing on contribution of interleukin-2 receptor (IL-2R) signaling, which is known to induce the signal necessary for the G_1/S transition in activated T cells [16–18], to the induction of PHGDH gene expression. Using antisense PHGDH oligonucleotide, the requirement of PHGDH for DNA synthesis of activated T cells was also investigated. We show that the expression of PHGDH mRNA is not detected in G_0 T cells but is induced in G_1 phase by IL-2R signaling and reaches a maximal level in S phase of activated T cells, along with a significant reduction in [^{3}H]TdR incorporation into DNA of activated T cells in the presence of antisense PHGDH oligonucleotide that can specifically block the translation of PHGDH transcript. These results first demonstrate that the expression of PHGDH gene depends on IL-2R signaling and is required for S phase during the proliferation of activated T cells.

2. Materials and methods

2.1. Animals

C57BL/6 male mice, 4–6 months old, were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and maintained in the Gerontology Research Center Animal Facility (Baltimore, MD, USA) and the Animal Resources Center at Korea Research Institute of Bioscience and Bioengineering (Daejeon, Korea) under specific-pathogen free conditions.

2.2. Chemicals, antibodies, reagents, cDNA probes, and medium

Phorbol dibutyrate (PBu_2) was purchased from Calbiochem (La Jolla, Ca, USA), and cyclosporine A (CsA) and nocodazole were obtained from Sigma Chemical (St. Louis, Mo, USA). Radioactive materials including [α-^{32}P]dCTP (≈3000 Ci/mmol), and random primer labeling kit were purchased from Amersham (Arlington Heights, IL, USA). [^{3}H]TdR (2 Ci/mmol) and Nylon membrane (GeneScreen PlusTM) for Northern analysis was obtained from New England Nuclear (Boston, MA, USA). Mouse recombinant IL-2 (rIL-2) was purchased from Genzyme (Cambridge, MA, USA). Rabbit anti-mouse IgG were obtained from Jackson ImmunoResearch (West Grove, PA, USA), and monoclonal anti-CD3 of mouse was purchased from PharMingen (San Diego, Ca, USA). Goat anti-mouse IL-2 antibody for neutralization of IL-2 activity was purchased from R&D systems (Minneapolis, MN, USA), and rat anti-mouse IL-2Rα neutralizing antibody was from Abcam (Cambridge, MA, USA). ECL kit was purchased from Amersham (Arlington, Heights, IL, USA). Immobilon-P transfer membrane for Western analysis was obtained from Millipore (Bedford, MA, USA). All reagents for electrophoresis were supplied by Sigma (St. Louis, MO, USA). The PHGDH antisense (AS-1, 5′-GCCATTGCTAG AGTCAG-3′; AS-2, 5′-CCAGGCACATGATCAT-3′; AS-3, 5′-GGACAGC TGATGACAT-3′; AS-4, 5′-GTGACATTGAGGCC-3′), and sense (S-1, 5′-CTGACTC-TAGCAATGGC-3′; S-2, 5′-ATGATCATGTGCCTGG-3′; S-3, 5′-ATGTCATCAGCTGTCC-3′; S-4, 5′-GGCCTCAATGTCAC-3′) phosphorothioate oligonucleotides were purchased from Oligos Etc. Inc. (Wilsonville, OR, USA). The plasmid pcEXVmIL-2-R8 containing the cDNA of murine IL-2R [19] was a gift from Dr. D. Pardoll (Johns Hopkins University, Baltimore, MD, USA). The plasmid pBluscript SK(±) containing the cDNA of murine homologue of cdc2 [20] was provided by Dr. J. Corden (Johns Hopkins University, Baltimore, MD, USA). The plasmid AMIL-2RP-26 containing the cDNA of murine IL-2Rβ [21] was obtained from Dr. T. Kono (Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan). The plasmid pHRR118 containing the cDNA of rat 18S rRNA [22] was obtained from Dr. Ira G. Wool (University of Chicago, IL, USA). The 40-mer DNA probe for murine IL-2 was purchased from Oncogene Science Inc. (Manhasset, NY, USA). The human PHGDH cDNA, which shows 93% sequence similarity with murine PHGDH cDNA [5], was used to detect murine PHGDH mRNA. Rabbit antiserum raised against recombinant human PHGDH protein that was expressed using *Escherichia coli* system was prepared essentially as previously described [14]. This polyclonal anti-human PHGDH was able to react with both human PHGDH and murine PHGDH. The culture medium used for activation of T lymphocytes was RPMI 1640 containing 10% FBS (HyClone, Logan Utah, USA), 20 mM HEPES, 5×10^{-5} M β-mercaptoethanol, and 100 μg/ml gentamycin, and is referred to as complete medium. The culture media deprived of either L-serine or L-serine and glycine were purchased from JBI (Daegu, Korea), and supplemented with 10% dialyzed FBS (JRH Biosciences, KS, USA). The culture medium for investigating the effect of antisense PHGDH oligonucleotide on activation of G_0 T cells was the RPMI 1640 medium deprived of L-serine, and contained 10% dialyzed FBS.

2.3. Cell culture, activation of T cells, and [^{3}H]TdR incorporation

The preparation and activation of murine resting (G_0) T cells were performed as previously described [23,24]. The recovered G_0 T cells were ≈95% Thy-1 positive with a CD4/CD8 ratio of ≈1 as determined by flow cytometry. The incorporation of [^{3}H] TdR into DNA by G_0T cells (10^5) activated with immobilized anti-CD3 was determined as described elsewhere [25]. For activation in the presence of synthetic oligonucleotides, G_0 T cells at a density of 2×10^6 cells/ml were pretreated with indicated amounts of antisense or sense PHGDH oligonucleotides at 37 °C in the culture medium. After 4 h, cells were cultured for 40 h in 6-well plates (BD Biosciences, Bedford, MA, USA) or 96-well plates previously coated with anti-CD3.

2.4. Flow cytometry analysis

Approximately 1×10^6 T cells were fixed with 67% cold ethanol for 1 h. The cells were washed with PBS, and resuspended with 12.5 μg RNase in 250 μl of 1.12% sodium citrate buffer (pH 8.45). Incubation was continued at 37 °C for 30 min before staining of the cellular DNA with 250 μl of propidium iodide (50 μg/ml) for 20 min. The stained cells were analyzed on a FACScan flow cytometer for relative DNA content, based on increased red fluorescence.

2.5. Northern blot analysis

Total RNA was extracted and isolated by solubilization in guanidine thiocyanate as described elsewhere [25]. Equivalent amounts