Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

M2b macrophage polarization accompanied with reduction of long noncoding RNA GAS5

Ichiaki Ito [a, *], Akira Asai [b], Sumihiro Suzuki [c], Makiko Kobayashi [a], Fujio Suzuki [a]

[a] Department of Internal Medicine, The University of Texas Medical Branch, Galveston, TX, USA
[b] The Second Department of Internal Medicine, Osaka Medical College, Takatsuki, Osaka, Japan
[c] Department of Biostatistics and Epidemiology, University of North Texas Health Science Center, Fort Worth, TX, USA

ARTICLE INFO

Article history:
Received 6 September 2017
Accepted 12 September 2017
Available online xxx

Keywords:
Macrophage polarization
Bone marrow-derived macrophages
Long noncoding RNA GAS5
Nonsense-mediated RNA decay

ABSTRACT

Macrophages (Mϕ) are highly plastic and change their functional phenotypes depending on microenvironmental signals. Recent studies have shown that microRNAs are involved in the polarization of Mϕ. In this study, we demonstrated that the phenotype of M2bMϕ [CCL1(+) IL-10(+) LIGHT(+)] switches to other phenotypes with interchangeability attained through the increased expression of growth arrest-specific 5 RNA (GAS5 RNA), a long noncoding RNA. GAS5 RNA has been described as a silencer of the CCL1 gene. Various phenotypes of Mϕ were prepared from bone marrow-derived Mϕ (BMDMϕ) after stimulation with IFNγ [M(IFNγ)/M1Mϕ], IL-4 [M(IL-4)/M2aMϕ], LPS and immobilized IgG [M(LPS + IC)/M2bMϕ], and IL-10 [M(IL-10)/M2cMϕ]. BMDMϕ cultured with medium [M(no)/quiescent Mϕ] were used as a control. As compared to M(no), M(IFNγ), M(IL-4) and M(IL-10), the reduced level of GAS5 RNA was shown in M(LPS + IC). CCL1 and LIGHT mRNAs (typical biomarkers of M2bMϕ) were not expressed by M(LPS + IC) transduced with a GAS5 gene using lentiviral vector. The reduction of GAS5 RNA in M(LPS + IC) was mediated by the activation of nonsense-mediated RNA decay (NMD) pathway. BMDMϕ overexpressed with GAS5 RNA after GAS5 gene transduction did not polarize to M2bMϕ even though they were stimulated with LPS and IC in combination. These results indicate that the reduction of GAS5 RNA influenced by the NMD pathway activation leads to the Mϕ polarization stimulated with LPS and IC in combination.

1. Introduction

Macrophages are highly plastic and heterogeneous [1]. Polarized Mϕ can be discriminated by their abilities to produce cytokines and gene expression profiles [2–4]. Generally, at least two types of Mϕ with distinct phenotypes are able to isolate from hosts with infections: M1Mϕ (IL-12$^+$IL-10$^-$ Mϕ) and M2Mϕ (IL-12$^-$IL-10$^+$ Mϕ) [1–4]. M1Mϕ are known as a major effector cell on the host antibacterial innate immunities [5,6]. However, M1Mϕ are not easily generated in hosts whose M2Mϕ predominate [7,8]. M2Mϕ inhibit the Mϕ polarization from interchangeable Mϕ to M1Mϕ [9–11]. M2Mϕ are phenotypically subdivided into three: M2aMϕ (CCL17$^+$CD206$^+$IL-10$^+$ Mϕ), M2bMϕ (CCL1$^+$LIGHT$^+$IL-10$^+$ Mϕ), and M2cMϕ (CXCL13$^+$ CD206$^+$IL-10$^+$ Mϕ) [1–4,7–11]. Based on their plasticity, M1Mϕ switch again to M2a/cMϕ through a certain miRNA expression involved in the stimulation of pathogen-associated molecular patterns [12]. Further, M2a/cMϕ switch yet again to a non-M2a/cMϕ provided that IL-4 or IL-10 is absent [13]. Therefore, the lifespan of M1Mϕ and M2a/cMϕ is relatively short. Because M2bMϕ produce CCL1 [14], an essential chemokine for prolonging their lifespan [15], they live longer without any exogenous growth factors. Thus, the susceptibility to gut bacteria-associated sepsis for hosts with M2bMϕ continues until the M2bMϕ disappear [8–11].

Noncoding RNAs are transcribed from eukaryotic genome with no protein-coding capacity [16] and have been demonstrated to play an important role on cell development, metabolism, differentiation, and homoeostasis [17]. In cells, noncoding RNAs have various molecular functions, including modulating transcriptional patterns, protein activities, and RNA processing [16,17]. Because Mϕ are highly plastic and have the ability to change their phenotypes under extracellular stimuli through altering transcriptional pattern [3]; noncoding RNAs are considered to modulate gene expressions

* Corresponding author. The University of Texas Medical Branch, Department of Internal Medicine, 301 University Boulevard, Galveston, TX, 77555-0435, USA.
E-mail address: icito@utmb.edu (I. Ito).

http://dx.doi.org/10.1016/j.bbrc.2017.09.053
0006-291X/

during the process of Mϕ polarization. Within noncoding RNA, miRNAs have been shown to play a key role in the polarization between M1 and M2Mϕ [12], but the role of long noncoding RNAs (lncRNAs) in Mϕ polarization is unclear. In this study, M2bMϕ were switched to other Mϕ phenotypes with interchangeability after expression of a lncRNA, growth arrest-specific 5 (GAS5). CCL1 gene expression was silenced in M2bMϕ transduced with the GAS5 gene. CCL1 has already been characterized as an essential chemokine for prolonging the life of M2bMϕ [15]. In our previous studies, M2bMϕ switched to quiescent Mϕ after treatment with CCL1 antisense ODN or anti-CCL1 antibody [8,10,11,15,18,19]. GAS5 RNA has been shown to suppress CCL1 gene expression in bladder cancer cells [20]. The RNA level of GAS5 was largely reduced in bone marrow-derived Mϕ (BMDMϕ) after stimulation with lipopolysaccharide (LPS) and immobilized IgG (immune complex, IC). These are a typical preparation for M2bMϕ abbreviated as M(LPS + IC) [21]. As compared to quiescent Mϕ [M(no)], however, the GAS5 RNA expression was not reduced in other various phenotypes of Mϕ created by the IFNγ stimulation [M(IFNγ)], IL-4 stimulation [M(IL-4)], and IL-10 stimulation [M(IL-10)]. Also, M(LPS + IC) transduced with the GAS5 gene using lentivirus vector lost their typical biomarkers for M2bMϕ, and BMDMϕ transduced with the GAS5 gene did not switch to M2bMϕ even though they were stimulated with LPS and IC in combination. The reduction of GAS5 in M(LPS + IC) was shown to be mediated by the activation of nonsense-mediated RNA decay (NMD) pathway. These results indicate that BMDMϕ polarized to M2bMϕ under the decreased GAS5 RNA expression influenced by the NMD pathway activation.

2. Materials and methods

2.1. Preparation and polarization of BMDMϕ

BMDMϕ were prepared from bone marrow cells, as previously described [22]. Briefly, bone marrow cells (5×10^5 cells/ml) isolated from BALB/c mouse femurs and tibias were cultured for 7 days in complete medium supplemented with 25 ng/ml of M-CSF on a Petri dish. The purity of $F4/80^+$ cells 7 days after the cultivation was routinely more than 98%. Cells (1×10^6 cells/ml) were harvested and recultured again in complete medium supplemented with 20 ng/ml of IFNγ [23], 20 ng/ml of IL-4 [24], or 50 ng/ml of IL-10 [25], respectively, for 24 h at 37 °C. Cells harvested were designed as M(IFNγ), M(IL-4), or M(IL-10) [21]. Culture plates were previously coated with 100 μg/ml of murine IgG for 2 h at 37 °C. With 100 ng/ml of LPS, M(LPS + IC) were prepared from BMDMϕ [14]. These Mϕ preparations were harvested, and total RNAs, extracted from these cells, were assayed for the expression of iNOS and IL-12 [for M(IFNγ)]; CD206, ARG1, and CCL17 [for M(IL-4)]; CCL1 and LIGHT [for M(LPS + IC)]; and ARG1 and CXCL13 [for M(IL-10)] by RT-PCR, as described below. For the knockdown experiment, Mϕ were transfected with siRNA specific for mouse UPF1 using Lipofectamine RNAi MAX according to the manufacturer's protocol. To test the interchangeability of Mϕ, cells were stimulated with 10 μg/ml of CpG DNA for 24 h, as previously described [26]. Obtained Mϕ were stained with anti-CD38 and intracellular IL-12 antibodies using Fixation/Permeabilization Solution Kit and analyzed by flow cytometry (BD LSRFortessa). Data were analyzed in FlowJo 10.2 software (Tree Star, Ashland, OR, USA).

2.2. Preparation of GAS5-expressing lentivirus

Murine GAS5 cDNA was amplified from pCMV-Sport6-GAS5 plasmid and cloned into pLenti7.3/V5-TOPO vector (pLenti7.3-GAS5). Lentiviruses were prepared using HEK293FT cells as described in the manufacturer's protocol. In brief, 3 μg of pLenti-GAS5 vector and 9 μg of packaging mix were cotransfected into HEK293FT cells using a transfection reagent Lipofectamine 2000. Seventy-two hours after transfection, supernatants were filtered (0.45-μm filter). To concentrate produced viruses in the supernatants, PEG-it Virus Precipitation Solution was added, and the mixture was incubated for 16 h at 4 °C. Virus in the mixture was precipitated by centrifugation at 1500 × g for 30 min, resuspended in PBS, and stored at −80 °C until further use. The virus suspension was titrated onto HEK293FT cells by flow cytometry using GFP expression from pLenti7.3/V5-TOPO vector. Mock viruses were generated by the same procedure using otherwise identical vector lacking GAS5 cDNA (Negative control lentivirus, NC lentivirus).

2.3. Statistical analyses

Data were expressed as mean ± SD. Data were analyzed using a Student's t-test. The results were considered to be significant if $p < 0.05$.

Materials and methods for mice, reagents, and media, Western blotting analysis, and gene expression analyses are found at Supplementary materials and methods.

3. Results

3.1. GAS5 RNA expression in various phenotypes of Mϕ

BMDMϕ were cultured with media [M(no)] or stimulated with IFNγ [M(IFNγ)], IL-4 [M(IL-4)], LPS and IC in combination [M(LPS + IC)], or IL-10 [M(IL-10)]. Twenty-four hours after stimulation, these Mϕ preparations were tested for their phenotypes by their specific biomarkers. As a result, M(IFNγ) were confirmed as M1Mϕ because they expressed iNOS and IL-12 but did not express CD206, ARG-1, CCL17, CCL1, LIGHT, or CXCL13 (Fig. 1). M(IL-4) were confirmed as M2aMϕ because they expressed CD206, ARG-1, and CCL17 but did not express iNOS, IL-12, CCL1, LIGHT, or CXCL13 (Fig. 1A). M(LPS + IC) were confirmed as M2bMϕ because they expressed CCL1 and LIGHT but did not express iNOS, IL-12, ARG-1, CD206, CCL17, or CXCL13 (Fig. 1A). M(IL-10) were confirmed as M2cMϕ because they expressed ARG-1 and CXCL13 but did not express iNOS, IL-12, CCL17, CCL1, or LIGHT (Fig. 1A). These Mϕ preparations were analyzed for the expression of GAS5 RNA by RT-PCR. As shown in Fig. 1A, GAS5 RNA was minimally expressed in M(LPS + IC), but it was abundantly expressed in M(no) and all other phenotypes of Mϕ. The expression levels of GAS5, iNOS, CCL17, CCL1, and CXCL13 in these Mϕ were also confirmed by real-time PCR (Fig. 1B). These results indicate that GAS5 RNA is not expressed by M(LPS + IC) but M(no), M(IFNγ), M(IL-4), and M(IL-10) are cells to express this RNA.

3.2. Effect of GAS5 gene transduction on the properties of M2bMϕ

The role of GAS5 in the cellular properties of M(LPS + IC) was examined. BMDMϕ stimulated with LPS and IC for 24 h were transduced with the GAS5 gene via GAS5-encoded lentiviral vector (GAS5 lentivirus). The results obtained are shown in Fig. 2. Although M(LPS + IC) expressed CCL1 mRNA (A), CCL1 mRNA level was reduced up to 80% in the same cells after transduction of the GAS5 gene (B). The same results were obtained (Fig. 2C and D) when the intracellular CCL1 and the CCL1 producing ability of M(LPS + IC) transduced with the GAS5 gene were tested. In response to CpG DNA (a typical M1Mϕ inducer), 80% or more of M(LPS + IC) exposed to GAS5 lentivirus switched to $CD38^+$ cells, while only 18% of M(LPS + IC) exposed to NC lentivirus switched to $CD38^+$ cells (Fig. 2E). These results indicate that M(LPS + IC) transduced with the GAS5 gene loose their specific biomarker for

Please cite this article in press as: I. Ito, et al., M2b macrophage polarization accompanied with reduction of long noncoding RNA GAS5, Biochemical and Biophysical Research Communications (2017), http://dx.doi.org/10.1016/j.bbrc.2017.09.053