Biochemical and Biophysical Research Communications 457 (2015) 445–450

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Pam2 lipopeptides systemically increase myeloid-derived suppressor cells through TLR2 signaling

Akira Maruyama [1], Hiroaki Shime [*, 1], Yohei Takeda, Masahiro Azuma, Misako Matsumoto, Tsukasa Seya [*]

Department of Microbiology and Immunology, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan

ARTICLE INFO

Article history:
Received 24 December 2014
Available online 13 January 2015

Keywords:
Myeloid-derive suppressor cells (MDSCs)
Pam2 lipopeptides
Toll-like receptor 2
Immunosuppression
Antitumor immunotherapy

ABSTRACT

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells that exhibit potent immunosuppressive activity. They are increased in tumor-bearing hosts and contribute to tumor development. Toll-like receptors (TLRs) on MDSCs may modulate the tumor-supporting properties of MDSCs through pattern-recognition. Pam2 lipopeptides represented by Pam2CSK4 serve as a TLR2 agonist to exert antitumor function by dendritic cell (DC)-priming that leads to NK cell activation and cytotoxic T cell proliferation. On the other hand, TLR2 enhances tumor cell progression/invasion by activating tumor-infiltrating macrophages. How MDSCs respond to TLR2 agonists has not yet been determined. In this study, we found intravenous administration of Pam2CSK4 systemically up-regulated the frequency of MDSCs in EG7 tumor-bearing mice. The frequency of tumor-infiltrating MDSCs was accordingly increased in response to Pam2CSK4. MDSCs were not increased by Pam2CSK4 stimuli in TLR2 knockout (KO) mice. Adoptive transfer experiments using CFSE-labeled MDSCs revealed that the TLR2-positive MDSCs survived long in tumor-bearing mice in response to Pam2CSK4 treatment. Since the increased MDSC population sustained immune-suppressive properties, our study suggests that Pam2CSK4-triggered TLR2 activation enhances the MDSC potential and suppress antitumor immune response in tumor microenvironment.

1. Introduction

TLR2 signaling pathway plays a critical role in induction of protective immunity against infection [1,2]. TLR2 enhances dendritic cell/macrophage functions that cause host defense, but exerts a controversial effect on cancer development [2]. Recent reports demonstrated that treatment with purified TLR2 ligands such as Pam2CSK4, Pam3CSK4, MALP2 or related synthetic compounds inhibited tumor growth in mice tumor implant models [3,4]. Pam2 lipopeptides trigger activation of TLR2 in combination with TLR6 or TLR1 in conventional DCs, which leads to maturation of the DCs through the MyD88-dependent signaling pathway, resulting in NK cell activation and CTL proliferation [5–7].

In tumor-bearing mice with systemic exposing to TLR2 agonists, however, an opposite effect was reported: TLR2 signal-induced inflammation may contribute to tumor progression. TLR2 is also expressed on immune cells with regulatory properties that include tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) as well as tumor cells [8–10]. Host cell-derived endogenous TLR2 ligand, such as versican, a chondroitin sulfate proteoglycan derived from cancer cells, stimulates macrophages to produce TNF-α, which enhances lung metastasis of cancer cells [11]. Furthermore, Pam2CSK4 primes DC activation to induce expansion of $Foxp3^{+}CD25^{+}CD4^{+}$ regulatory T cells (Treg) and cause immune tolerance against cancer [12,13]. These reports suggest that TLR2 signaling may modulate the myeloid cell function, which promotes growth, invasion, or metastasis of tumor cells. There might be cell type-to-cell type difference in TLR2 response to its ligands, which critically determines their mode for regulation against tumor progression or survival.

MDSCs are heterogenous populations of immature myeloid cells that have immunosuppressive activity. MDSCs are expanded in

Abbreviations: CFSE, carboxyfluorescein succinimidyl ester; CTL, cytotoxic T lymphocyte; DAMP, damage-associated molecular pattern; DC, dendritic cell; MALP-2, macrophage activating lipopeptide-2; NK, natural killer; Pam2, 16 S-[2,3-bis(palmitoyl)propyl]cysteine; TLR, toll-like receptor.

* Corresponding authors. Fax: +81 11 706 7866.
E-mail addresses: shime@med.hokudai.ac.jp (H. Shime), seya-tu@pop.med.hokudai.ac.jp (T. Seya).

[1] The first two authors were equally contributed.

http://dx.doi.org/10.1016/j.bbrc.2015.01.011
0006-291X/

tumor-bearing mice and patients with cancer such as colon cancer, bladder cancer, lung cancer, and ovarian cancer. They impede the efficacy of cancer immunotherapy [14]. Depletion of MDSCs augments anti-tumor activity of host immune cells by restoring effector cell function [15]. Mouse MDSCs are characterized by the markers of $CD11b^{+}Gr1^{+}$. MDSCs subvert anti-tumor immunity by suppression of DC maturation, T cell proliferation and NK cell activation, and by induction of immunosuppressive M2 macrophage and Tregs [14,16,17]. MDSCs consist of $Ly6C^{high}Ly6G^{-}CD11b^{+}$ monocytic MDSCs (M-MDSCs) and $Ly6C^{low}Ly6G^{+}CD11b^{+}$ granulocytic MDSCs (G-MDSCs). Both subsets show distinct features and exert immunosuppressive activity by different mechanisms [18]. Inflammation-associated molecules induce accumulation of MDSCs and enhance immunosuppressive activity in local environment. Recent reports demonstrated that TLR signaling regulated tumor growth by modifying MDSC function [19]. MDSCs express TLRs, through which TLR ligands modify their accumulation, differentiation and function [20]. Tumor cell-derived exosomes containing Hsp72 induce expansion and suppressive activity of MDSCs through the TLR2-IL-6-STAT3 axis [9]. The S100A8/A9 complex produced in tumor regulates accumulation and suppressive activity of MDSCs through the TLR4 signaling pathway [21,22]. On the other hand, TLR9 and TLR3 ligands such as CpG ODN and poly I:C, respectively, are demonstrated to modify MDSC function directly or indirectly. Those functionally modified MDSCs exhibit loss of immunosuppressive activity against T cell function as well as act as the accessory cells for NK cell activation [23–25]. In this context, what happens in MDSCs when TLR2 agonist is exogenously administered to tumor-bearing mice remains poorly understood.

In this study, we revealed that Pam2CSK4 induces accumulation of MDSCs in spleen and tumor in tumor-bearing hosts. Pam2CSK4 can support long survival of MDSCs through the TLR2 signaling pathway.

2. Materials and methods

2.1. Mice and cells

C57BL/6J (B6 WT) female mice were obtained from CLEA Japan Inc. (Tokyo, Japan). $TLR2^{-/-}$ mice were provided by Dr. Shizuo Akira (Osaka University, Osaka, Japan). C57BL6-Tg (CAG-EGFP) mice (EGFP transgenic mice) were provided by Dr. Masaru Okabe (Osaka University). The mice were maintained in the Hokkaido University Animal Facility (Sapporo, Jappan). *Mice of 8- to 12-weeks of age* were used in all experiments that were performed according to animal experimental ethics committee guidelines of Hokkaido University. EG7 cells were purchased from ATCC and cultured in RPMI1640/10% FCS/55 μM 2-ME/1 mM sodium pyruvate/penicillin/streptomycin. B16D8 cells were established in our laboratory and cultured in RPMI1640/10% FCS/penicillin/streptomycin [26].

2.2. Reagents and antibodies

FITC-conjugated anti-CD45 (30-F11), Alexa-700 or APC-conjugated anti-CD45.2 (104), Alexa 700, FITC or PE-conjugated anti-CD11b (M1/70), biotinylated, APC-conjugated anti-Gr-1 (RB6-8C5), purified anti-CD16/CD32 (2.4G2), and isotype antibodies were obtained from Biolegend (San Diego, CA, USA). 2,3-bis (palmitoyl) propyl CSKKKK (Pam2CSK4) was synthesized by Biologica Co. Ltd (Nagoya, Japan). To rule out LPS contamination, we treated Pam2CSK4 with 200 μg/ml of polymixin B for 30 min at 37 °C before use.

2.3. Tumor models

Mice were shaved at the back and injected subcutaneously (s.c.) with EG7 cells (1×10^6) or B16D8 (6×10^5) suspended in 200 μl PBS(−). When tumor grew, tumor size was measured using a caliper. In some experiments, Pam2CSK4 was i.p. injected into tumor-bearing mice. Tumor volume was calculated using the following formula: tumor volume (cm^3) = (long diameter) × (short diameter)^2 × 0.4. Pam2CSK4 was injected intravenously (i.v.) as indicated.

2.4. Isolation of cells

Tumor-infiltrating myeloid cells were defined by gating in FACS-sorting as previously described [25,27]. $CD11b^{+}Gr1^{+}$ MDSCs were separated with anti-Gr-1 biotinylated antibody and streptavidin microbeads (Miltenyi Biotech) from spleen cell suspensions of EG7 tumor-bearing mice. The purity of isolated cells was more than 90% as assessed by flow cytometory. Almost 100% of $Gr1^{+}$ cells were $CD11b^{+}$.

2.5. Flow cytometric analysis

Cells prepared from mouse spleen, blood or tumor were blocked with anti-CD16/32 antibody and stained with fluorescent antibodies. Samples were analyzed with the FACS Calibur instrument or the FACS Aria II instrument (BD Bioscience) and data analysis was performed by the Flow Jo software (Tree Star, USA).

2.6. Cell proliferation assay

T cell proliferation was measured by changes in fluorescence intensity using CFSE. OT-I splenocytes were labeled with 1 μM CFSE for 10 min and cultured with CD11b + Gr1 + MDSC in the presence of 50 nM SL8 peptide ($OVA_{257\text{-}264}$) and/or 100 nM Pam2CSK4. After 3 days, cells were harvested, stained with APC-anti-CD8α and PE-anti-TCR vβ 5.1, 5.2 or Alexa 700-anti-CD3ε, and CFSE signal of the gated lymphocytes was analyzed with a FACS Calibur instrument or FACS Aria II instrument. The extent of cell proliferation was quantified by Flow Jo software.

2.7. Adoptive transfer

EG7 tumor-bearing mice were injected i.v. with 5×10^6 CFSE-labeled MDSCs, and then injected i.v. with 50 nmol Pam2CSK4. After 24 h, spleen cells were blocked with anti-CD16/32 antibody and stained with fluorescent antibodies. Samples were measured by flow cytometry using the FACS Aria II. Data analysis was performed using the Flow Jo software.

2.8. Statistics

If not otherwise stated, data were expressed as arithmetic means ± SD, and statistical analyses were made by 2-tailed Student's t test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Expansion of TLR2-expressing MDSCs in EG7 tumor-bearing mice

We examined TLR2 expression on MDSCs in C57BL6/J mice subcutaneously (s.c.) implanted with EG7 lymphoma cells. 21 days after tumor inoculation when tumor volumes reached 4–8 cm^3, spleen cells of the tumor-bearing mice were analyzed by flow

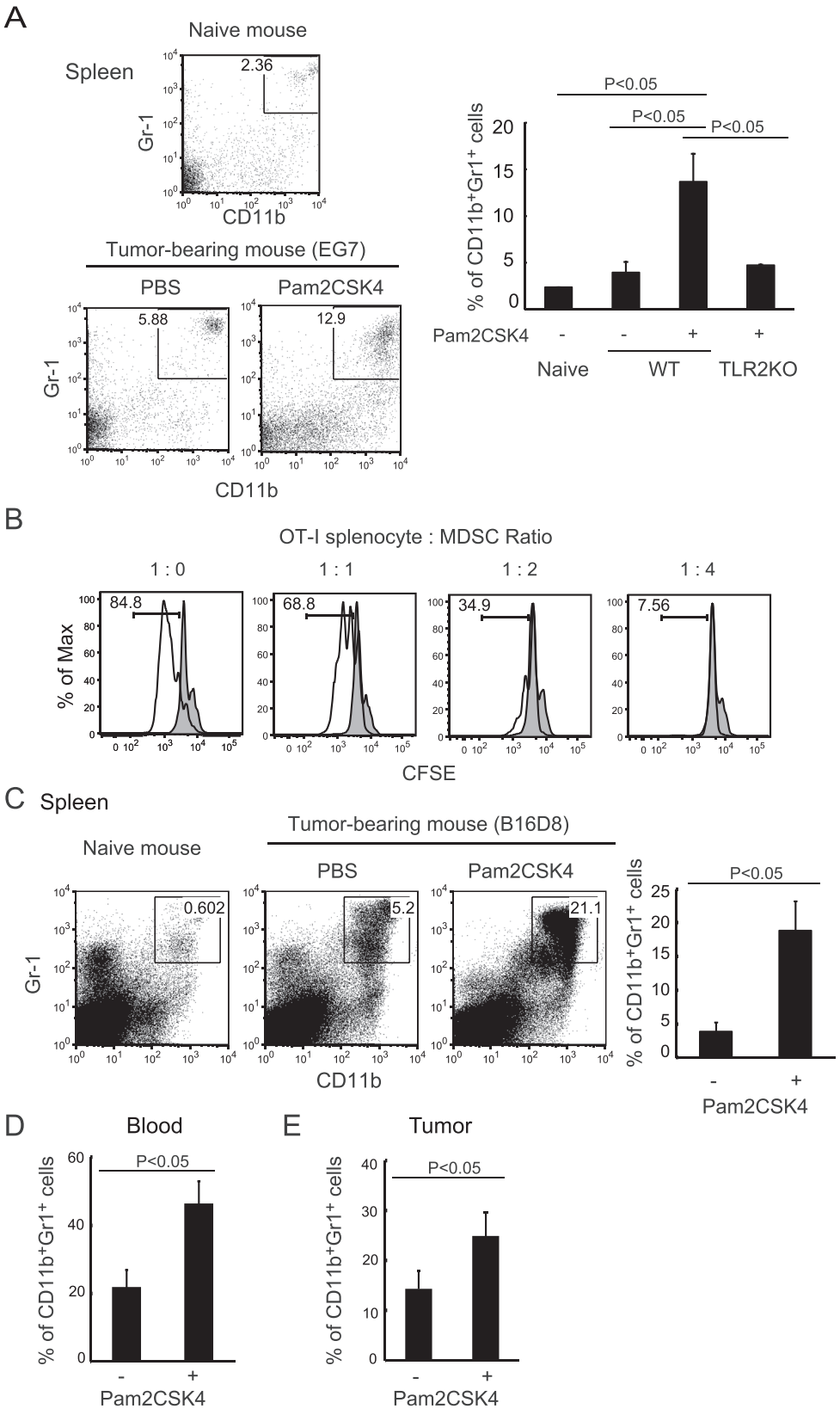

Fig. 1. Pam2CSK4 treatment induces accumulation of $CD11b^+Gr1^+$ MDSCs in tumor-bearing mice through TLR2 signaling. EG7 lymphoma cells (1×10^6) were implanted s.c. into B6 WT mice (A, B, and D), TLR2 KO mice (A) or EGFP transgenic mice (E) as described in materials and methods. B16D8 cells (6×10^5) were implanted s.c. into B6 WT mice (C). When tumor size was reached between 1 and 2.5 cm^3 (23–28 days after inoculation), mice were injected i.v. with PBS or 50 nmol Pam2CSK4. After 48 h, spleens (A and C), peripheral blood (D), and tumors (E) were isolated and the frequency of $CD11b^+Gr1^+$ cells in $CD45^+$ cells (A, C, and D) or in GFP^+CD45^+ cells (E) was determined by flow cytometory. Data shown in the graph represent mean ± SD. n = 3. Numbers in the graph represent the percentage of gated cells. In (B), $CD11b^+Gr1^+$ cells were isolated from EG7 tumor-bearing mice and analyzed suppressive activity on OT-I T cell proliferation as described in materials and methods. The CFSE histograms are gated for $CD8\alpha^+$TCR vβ 5.1, 5.2^+ cells. Open or closed histograms represent the cells cultured in the presence or absence of SL8 peptides, respectively. The numbers indicate the percentage of proliferated cells in open histograms.

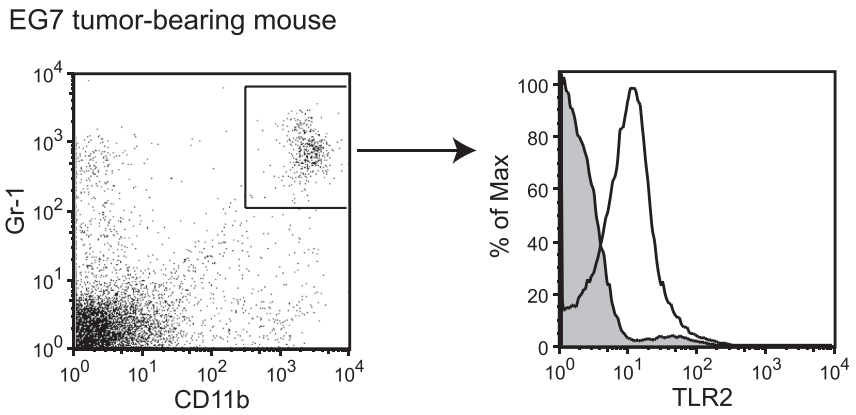

Fig. 2. CD11b^{+}Gr1^{+} MDSCs express TLR2 on their surface. TLR2 expression level on CD11b^{+}Gr1^{+} cells in spleen of EG7 tumor-bearing mice (21 days after tumor challenge) was analyzed by flow cytometory. The open histogram represents staining with anti-TLR2 antibody and the shaded histogram represent staining with isotype control antibody.

cytometory. The frequency of CD11b^{+}Gr1^{+} cells in spleens was significantly increased in EG7 tumor-bearing mice compared with tumor-free mice (Fig. 1A). CD11b^{+}Gr1^{+} cells harvested from tumor-bearing mice treated with Pam2CSK4 suppressed antigen-dependent T cell proliferation in a dose-dependent manner when cocultured with OT-I splenocytes, demonstrating that this population had MDSC-like activity (Fig. 1B). We found that TLR2 was highly expressed in CD11b^{+}Gr1^{+} MDSCs in spleen judged by flow cytometoric analysis (Fig. 2). Thus, our results indicate that TLR2-expressing MDSCs are expanded in EG7 tumor-bearing mice.

3.2. *Pam2CSK4 treatment induces accumulation of MDSCs in tumor-bearing mice through TLR2-dependent mechanism*

To examine whether TLR2 activation by Pam2CSK4 affects accumulation of MDSCs *in vivo*, we analyzed the proportion of CD11b^{+}Gr1^{+} cells in tumor-bearing mice after Pam2CSK4 i.v. injection. We measured the percentage of CD11b^{+}Gr1^{+} cells in spleens in EG7 tumor-bearing mice 48 h after Pam2CSK4 administration. Although the proportion of CD45^{+} cells in spleen was not altered (85 ± 6.07% of PBS-treated mice vs 87.3 ± 7.11% of Pam2CSK4-treated mice), Pam2CSK4 administration significantly increased the percentage of CD11b^{+}Gr1^{+} cells in CD45^{+} cells of spleens in EG7 tumor-bearing mice (Fig. 1A). CD11b^{+}Gr1^{+} cells harvested from the spleens of Pam2CSK4-treated tumor-bearing mice suppressed T cell proliferation in a dose-dependent manner when the cells were co-cultured with OT-1 splenocytes, demonstrating that this myeloid population had MDSC-like activity (Fig. 1B). Similar results were obtained with spleen CD11b^{+}Gr1^{+} cells in B16-implanted mice (Fig. 1C, data not shown). The increased percentage of CD11b^{+}Gr1^{+} cells was not observed after Pam2CSK4 treatment in TLR2$^{-/-}$ mice implanted with EG7 tumor (Fig. 1A). Pam2CSK4 facilitated systemic increases of CD11b^{+}Gr1^{+} MDSCs: incremental MDSCs were confirmed in spleens, circulating blood and tumors. The percentage of CD11b^{+}Gr1^{+} cells in peripheral blood was increased in peripheral blood in response to Pam2CSK4 treatment (Fig. 1D). To examine whether MDSCs accumulate in tumor, we used EGFP transgenic mice to distinguish host-derived CD45^{+} cells from EG7 cells which also express CD45. Although the proportion of GFP-positive cells in tumor was barely changed, the percentage of CD11b^{+}Gr1^{+} cells in GFP-positive cells was increased 48 h after Pam2CSK4 treatment (Fig. 1E). Thus, these results suggest that TLR2 activation induced by Pam2CSK4 leads to systemic accumulation of MDSCs rather than organ-specific recruitment of MDSCs in tumor-bearing mice.

3.3. *Pam2CSK4 treatment supports survival of MDSCs in tumor-bearing mice*

MDSCs are a short-lived cell population which shows rapid turnover in tumor-bearing mice [28]. We examined whether Pam2CSK4 prolonged survival of MDSCs *in vivo*. MDSCs were

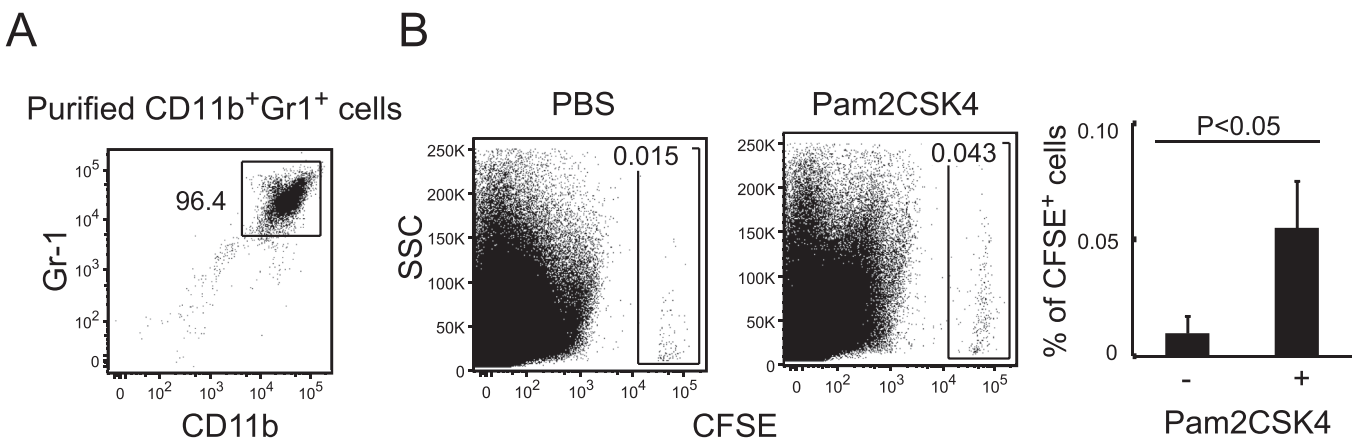

Fig. 3. Pam2CSK4 supports survival of adoptive transferred CD11b^{+}Gr1^{+} cells in tumor-bearing mice. CD11b^{+}Gr1^{+} cells (A) isolated from spleens of EG7 tumor-bearing mice were labeled with CFSE and adoptively transferred into EG7 tumor-bearing mice. The mice were injected i.v. with PBS or 50 nmol Pam2CSK4. After 24 h, spleen cells were analyzed by flow cytometory (B). The frequency of CFSE-positive cells was determined. The numbers indicate the percentage of CFSE-positive cells in CD45^{+}-gated splenocytes.

isolated from the spleen of tumor-bearing mice and labeled with CFSE, and then adoptively transferred into EG7 tumor-bearing mice. Treatment with poly I: C increased percentage of remaining CFSE-positive cells in CD45-positive splenocytes of EG7 tumor-bearing mice (Fig. 3A and B). Thus, our results suggest that Pam2CSK4 may support survival of MDSCs in tumor-bearing mice through the TLR2-dependent signaling pathway. CFSE-positive cells barely proliferated in the bone marrow within our setting (data not shown).

4. Discussion

Although TLR2 ligands can induce tumor regression by inducing anti-tumor immune responses mediated by DCs, cytotoxic T lymphocytes and NK cells, their effects on immunosuppressive cells including MDSCs have not been fully investigated. The purpose of this study was to determine the role of TLR2 signaling on accumulation of immunosuppressive MDSCs in tumor-bearing hosts. Our findings revealed that Pam2CSK4-induced TLR2 signaling enhances systemic expansion of MDSCs *in vivo*. Since MDSCs have strong immunosuppressive activity against anti-tumor immunity, our results suggest that treatment with TLR2 ligands may lead to augmentation of immunosuppression in tumor-bearing hosts.

MDSCs consist of two major subsets of M-MDSCs and G-MDSCs, both of which express TLR2. They show distinct morphology and differential mechanisms for immunosuppressive profiles. G-CSF, GM-CSF, and M-CSF are known as key growth factors for the regulation of survival, proliferation, and differentiation of MDSC subsets [29,30]. G-CSF or GM-CSF supports the survival of G-MDSCs in vitro [31]. TLR2 stimulation induces the production of these growth factors [32]. Intracellular signaling triggered by these growth factors contribute to the proliferation and survival of immature myeloid cells and prevent their differentiation to mature cells, resulting in accumulation of MDSCs. However, second signal induced by prolinflamamtory cytokines or TLR ligands are required to acquire immunosuppressive function [29]. TLR2 signal also induces the production of proinflammatory cytokines such as IL-6 and TNF-α by myeloid cells. A previous report demonstrated that TLR2 signal-induced IL-6 production was responsible for the development and survival of MDSCs through STAT3 activation [9]. TNF receptor signaling promotes the survival and accumulation of MDSCs [33]. S100A8/A9, which are produced by TLR2 signal activation, regulates the accumulation of MDSCs [34]. Thus, TLR2 signal may support survival and differentiation of MDSCs by inducing production of these cytokines in inflammatory milieu. TLR2 activation also induces proliferation of cancer cells by up-regulating the expression of numerous cell cycle progression and cell survival/anti-apoptosis genes [10], suggesting that TLR signal may directly induce survival or proliferation of MDSCs. Further analysis is required to identify the mechanisms that support MDSC accumulation by activating TLR2 signal.

MDSCs have strong immunosuppressive activity against CTLs, NK cells and DCs by producing immunosuppressive factors including arginase, TGF-β, reactive oxygen species (ROS), reactive nitrogen species (RNS), and IL-10. MDSCs also induce Tregs by producing arginase and/or IL-10 [14]. It remains unclear whether Pam2CSK4 influences immunosuppressive functions of MDSCs. In fact, Pam2CSK4 induces IL-10 and ROS production by DCs and macrophages through TLR2 signaling [35]. Therefore, Pam2CSK4 may not only support the survival but also regulate the immunosuppressive activity of MDSCs because the production of these molecules is tightly regulated by TLR2 signaling.

The regulatory mechanism of MDSC accumulation seems to be important for development of the effective therapeutic strategies to control these cells. MDSCs are produced in response to tumor-derived factors such as cytokines, chemokines, DAMPs, or microenvironmental factors such as hypoxia. Some of those are also provided by immune cells activated by endogenous ligand-induced TLR signaling. Our results suggest that MDSCs accumulate in tumor-bearing hosts in response to exogenously added TLR2 ligands. Adjuvant immunotherapy for cancer using TLR2 ligands has been proposed and some clinical trials are in progress [36]. Our results, however, unveiled the negative effects of TLR2 ligands on tumor immunity in terms of MDSC frequencies. Several reports demonstrated that the frequency of MDSCs is correlated with tumor size in several mouse models. MDSCs are frequently observed in patients with advanced cancer. Thus, TLR2 signal-induced accumulation of MDSCs may be critical for determining of success in immunotherapy against advanced cancer. The quality and properties of MDSCs have to be changed in TLR2 adjuvant therapy as in previous reports [25,27]. This point needs to be taken into consideration prior to the development of antitumor immunotherapy for cancer.

Conflict of interest statement

The authors have no conflict of interest.

Acknowledgments

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, and Culture (MEXT), “the Carcinogenic Spiral” a MEXT Grant-in-Project, the Ministry of Health, Labor, and Welfare of Japan, the Takeda Foundation, and the Kato Memorial Bioscience Foundation.

References

[1] T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on toll-like receptors, Nat. Immunol. 11 (2010) 373–384, http://dx.doi.org/10.1038/ni.1863.
[2] A. Mantovani, P. Allavena, A. Sica, F. Balkwill, Cancer-related inflammation, Nature 454 (2008) 436–444, http://dx.doi.org/10.1038/nature07205.
[3] T. Akazawa, H. Masuda, Y. Saeki, M. Matsumoto, K. Takeda, K. Tsujimura, et al., Adjuvant-mediated tumor regression and tumor-specific cytotoxic response are impaired in MyD88-deficient mice, Cancer Res. 64 (2004) 757–764.
[4] T. Seya, H. Shime, T. Ebihara, H. Oshiumi, M. Matsumoto, Pattern recognition receptors of innate immunity and their application to tumor immunotherapy, Cancer Sci. 101 (2010) 313–320, http://dx.doi.org/10.1111/j.1349-7006.2009.01442.x.
[5] M. Azuma, R. Sawahata, Y. Akao, T. Ebihara, S. Yamazaki, M. Matsumoto, et al., The peptide sequence of diacyl lipopeptides determines dendritic cell TLR2-mediated NK activation, PLoS ONE 5 (2010), http://dx.doi.org/10.1371/journal.pone.0012550.
[6] R. Sawahata, H. Shime, S. Yamazaki, N. Inoue, T. Akazawa, Y. Fujimoto, et al., Failure of mycoplasma lipoprotein MALP-2 to induce NK cell activation through dendritic cell TLR2, Microbes Infect. 13 (2011) 350–358, http://dx.doi.org/10.1016/j.micinf.2010.12.003.
[7] K.-Y. Shen, Y.-C. Song, I.-H. Chen, C.-H. Leng, H.-W. Chen, H.-J. Li, et al., Molecular mechanisms of TLR2-mediated antigen cross-presentation in dendritic cells, J. Immunol. 192 (2014) 4233–4241, http://dx.doi.org/10.4049/jimmunol.1302850.
[8] T.A. Wynn, A. Chawla, J.W. Pollard, Macrophage biology in development, homeostasis and disease, Nature 496 (2013) 445–455, http://dx.doi.org/10.1038/nature12034.
[9] F. Chalmin, S. Ladoire, G. Mignot, J. Vincent, M. Bruchard, J.-P. Remy-Martin, et al., Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells, J. Clin. Invest 120 (2010) 457–471, http://dx.doi.org/10.1172/JCI40483.
[10] H. Tye, C.L. Kennedy, M. Najdovska, L. McLeod, W. McCormack, N. Hughes, et al., STAT3-driven upregulation of TLR2 promotes gastric tumorigenesis independent of tumor inflammation, Cancer Cell. 22 (2012) 466–478, http://dx.doi.org/10.1016/j.ccr.2012.08.010.
[11] S. Kim, H. Takahashi, W.-W. Lin, P. Descargues, S. Grivennikov, Y. Kim, et al., Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis, Nature 457 (2009) 102–106, http://dx.doi.org/10.1038/nature07623.
[12] R.P.M. Sutmuller, M.H.M.G.M. den Brok, M. Kramer, E.J. Bennink, L.W.J. Toonen, B.-J. Kullberg, et al., Toll-like receptor 2 controls expansion and

function of regulatory T cells, J. Clin. Invest 116 (2006) 485–494, http://dx.doi.org/10.1172/JCI25439.
[13] S. Yamazaki, K. Okada, A. Maruyama, M. Matsumoto, H. Yagita, T. Seya, TLR2-dependent induction of IL-10 and Foxp3+ CD25+ CD4+ regulatory T cells prevents effective anti-tumor immunity induced by Pam2 lipopeptides in vivo, PLoS ONE 6 (2011) e18833, http://dx.doi.org/10.1371/journal.pone.0018833.
[14] D.I. Gabrilovich, S. Ostrand-Rosenberg, V. Bronte, Coordinated regulation of myeloid cells by tumours, Nat. Rev. Immunol. 12 (2012) 253–268, http://dx.doi.org/10.1038/nri3175.
[15] M.K. Srivastava, L. Zhu, M. Harris-White, U. Kar, U.K. Kar, M. Huang, et al., Myeloid suppressor cell depletion augments antitumor activity in lung cancer, PLoS ONE 7 (2012) e40677, http://dx.doi.org/10.1371/journal.pone.0040677.
[16] B. Huang, P.-Y. Pan, Q. Li, A.I. Sato, D.E. Levy, J. Bromberg, et al., Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host, Cancer Res. 66 (2006) 1123–1131, http://dx.doi.org/10.1158/0008-5472.CAN-05-1299.
[17] P. Serafini, S. Mgebroff, K. Noonan, I. Borrello, Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells, Cancer Res. 68 (2008) 5439–5449, http://dx.doi.org/10.1158/0008-5472.CAN-07-6621.
[18] J.-I. Youn, S. Nagaraj, M. Collazo, D.I. Gabrilovich, Subsets of myeloid-derived suppressor cells in tumor-bearing mice, J. Immunol. 181 (2008) 5791–5802.
[19] S.K. Bunt, V.K. Clements, E.M. Hanson, P. Sinha, S. Ostrand-Rosenberg, Inflammation enhances myeloid-derived suppressor cell cross-talk by signaling through toll-like receptor 4, J. Leukoc. Biol. 85 (2009) 996–1004, http://dx.doi.org/10.1189/jlb.0708446.
[20] S. Ostrand-Rosenberg, P. Sinha, Myeloid-derived suppressor cells: linking inflammation and cancer, J. Immunol. 182 (2009) 4499–4506, http://dx.doi.org/10.4049/jimmunol.0802740.
[21] P. Sinha, C. Okoro, D. Foell, H.H. Freeze, S. Ostrand-Rosenberg, G. Srikrishna, Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells, J. Immunol. 181 (2008) 4666–4675, http://dx.doi.org/10.4049/jimmunol.181.7.4666.
[22] P. Cheng, C.A. Corzo, N. Luetteke, B. Yu, S. Nagaraj, M.M. Bui, et al., Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein, J. Exp. Med. 205 (2008) 2235–2249, http://dx.doi.org/10.1084/jem.20080132.
[23] C. Zoglmeier, H. Bauer, D. Nörenberg, G. Wedekind, P. Bittner, N. Sandholzer, et al., CpG blocks immunosuppression by myeloid-derived suppressor cells in tumor-bearing mice, Clin. Cancer Res. 17 (2011) 1765–1775, http://dx.doi.org/10.1158/1078-0432.CCR-10-2672.
[24] Y. Shirota, H. Shirota, D.M. Klinman, Intratumoral injection of CpG oligonucleotides induces the differentiation and reduces the immunosuppressive activity of myeloid-derived suppressor cells, J. Immunol. 188 (2012) 1592–1599, http://dx.doi.org/10.4049/jimmunol.1101304.
[25] H. Shime, A. Kojima, A. Maruyama, Y. Saito, H. Oshiumi, M. Matsumoto, et al., Myeloid-derived suppressor cells confer tumor-suppressive functions on natural killer cells via polyinosinic: polycytidylic acid treatment in mouse tumor models, J. Innate Immun. 6 (2014) 293–305, http://dx.doi.org/10.1159/000355126.
[26] T. Akazawa, T. Ebihara, M. Okuno, Y. Okuda, M. Shingai, K. Tsujimura, et al., Antitumor NK activation induced by the toll-like receptor 3-TICAM-1 (TRIF) pathway in myeloid dendritic cells, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 252–257, http://dx.doi.org/10.1073/pnas.0605978104.
[27] H. Shime, M. Matsumoto, H. Oshiumi, S. Tanaka, A. Nakane, Y. Iwakura, et al., Toll-like receptor 3 signaling converts tumor-supporting myeloid cells to tumoricidal effectors, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 2066–2071, http://dx.doi.org/10.1073/pnas.1113099109.
[28] Y. Sawanobori, S. Ueha, M. Kurachi, T. Shimaoka, J.E. Talmadge, J. Abe, et al., Chemokine-mediated rapid turnover of myeloid-derived suppressor cells in tumor-bearing mice, Blood 111 (2008) 5457–5466, http://dx.doi.org/10.1182/blood-2008-01.
[29] T. Condamine, D.I. Gabrilovich, Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function, Trends Immunol. 32 (2011) 19–25, http://dx.doi.org/10.1016/j.it.2010.10.002.
[30] N. Sonda, M. Chioda, S. Zilio, F. Simonato, V. Bronte, Transcription factors in myeloid-derived suppressor cell recruitment and function, Curr. Opin. Immunol. 23 (2011) 279–285, http://dx.doi.org/10.1016/j.coi.2010.12.006.
[31] J.I. Youn, M. Collazo, I.N. Shalova, S.K. Biswas, D.I. Gabrilovich, Characterization of the nature of granulocytic myeloid-derived suppressor cells in tumor-bearing mice, J. Leukoc. Biol. 91 (2012) 167–181, http://dx.doi.org/10.1189/jlb.0311177.
[32] R.L. He, J. Zhou, C.Z. Hanson, J. Chen, N. Cheng, R.D. Ye, Serum amyloid A induces G-CSF expression and neutrophilia via toll-like receptor 2, Blood 113 (2009) 429–437, http://dx.doi.org/10.1182/blood-2008-03-139923.
[33] X. Zhao, L. Rong, X. Zhao, X. Li, X. Liu, J. Deng, et al., TNF signaling drives myeloid-derived suppressor cell accumulation, J. Clin. Invest 122 (2012) 4094–4104, http://dx.doi.org/10.1172/JCI64115.
[34] S.P. Hu, C. Harrison, K. Xu, C.J. Cornish, C.L. Geczy, Induction of the chemotactic S100 protein, CP-10, in monocyte macrophages by lipopolysaccharide, Blood 87 (1996) 3919–3928.
[35] T. Kawai, S. Akira, Toll-like receptors and their crosstalk with other innate receptors in infection and immunity, Immunity 34 (2011) 637–650, http://dx.doi.org/10.1016/j.immuni.2011.05.006.
[36] E. Vacchelli, A. Eggermont, C. Sautès-Fridman, J. Galon, L. Zitvogel, G. Kroemer, et al., Trial watch: toll-like receptor agonists for cancer therapy, Oncoimmunol 2 (2013) e25238, http://dx.doi.org/10.4161/onci.25238.