HUMAN VACCINES & IMMUNOTHERAPEUTICS
2018, VOL. 14, NO. 2, 285–287
https://doi.org/10.1080/21645515.2017.1395995

Taylor & Francis
Taylor & Francis Group

COMMENTARY

OPEN ACCESS Check for updates

Effects of STING stimulation on macrophages: STING agonists polarize into "classically" or "alternatively" activated macrophages?

Takayuki Ohkuri[†], Akemi Kosaka[†], Toshihiro Nagato, and Hiroya Kobayashi

Department of Pathology, Asahikawa Medical University, Asahikawa, Hokkaido, Japan

ABSTRACT

Stimulator of interferon genes (STING) was defined as an important molecule for promoting antitumor immunity through mediating type I interferon (IFN) production by sensing its ligands such as cyclic GMP-AMP (cGAMP). Our recent study indeed demonstrated that intratumoral injection of cGAMP showed effective antitumor responses via accumulating activated macrophages in the tumor microenvironment in a STING-dependent manner. Because the antitumor effect of cGAMP was abrogated when macrophages were depleted, the existence of the activated macrophages in the tumor site would be important for effective antitumor immune responses. Macrophages show phenotypic diversity and plasticity and are categorized into several groups by stimulation factors, e.g. IFN-γ and IL-4 for M1 and M2 macrophages, respectively. However, the impact of STING stimulation on the macrophage activation status remains to be evaluated. Here we summarize the complex polarized status of macrophages and the signaling cascade triggered by STING stimulation and also discuss the impact of STING signaling on the macrophage activation status for future directions.

ARTICLE HISTORY
Received 20 September 2017
Accepted 19 October 2017

KEYWORDS
STING; macrophage polarization; cGAMP; cancer immunotherapy; tumor microenvironment

Introduction

STING, which is a four-transmembrane protein localized in the endoplasmic reticulum (ER) and mitochondria-associated ER membrane, plays an important role of an adaptor in inducing type I IFNs following sensing of cyclic dinucleotides (CDN), i.e., bacteria-derived c-di-GMP and c-di-AMP and cyclic GMP-AMP (cGAMP) generated from intracellularly located viral or host DNAs including necrotic tumor cells by cGAMP synthase (cGAS).[1,2]

Macrophages are usually divided into two categories: (1) M1 or classically and (2) M2 or alternatively activated macrophages due to the type of stimulant factors.[3] M1 macrophages are generally stimulated with IFN-γ and/or LPS, which activate JAK-STAT1 signaling pathway to provide an anti-tumor phenotype with production of nitric oxide (NO) and pro-inflammatory cytokines such as TNF-α, IL-6, and IL-12. Whereas, M2 macrophages are involved in tissue repair and immunosuppressive functions and polarized by IL-4, IL-13, and other factors.[3–5] With regard to the widespread use of terms and definitions of macrophage as above, a common framework for nomenclature of macrophage status is recently proposed.[6]

STING signaling pathway and its contributions to antitumor immunity

STING activates several transcription factors such as NF-κB, IRF3, IRF7, and STAT6 to enhance inflammatory responses after receiving STING ligands. NF-κB and IRF3 induce type I IFNs to exert a potent antiviral immunity.[1] IRF7 is reported to contribute to the efficacy of DNA vaccines by inducing potent cytokine production in a STING-dependent manner.[7] Whereas, phosphorylated STAT6 by STING activation results in production of CCL2 and CCL20 to enhance antiviral innate immunity.[8] STING was initially identified as a key molecule for protecting hosts from bacterial and virus infections.[9,10] More recently, a role of STING in the field of antitumor immunity has been assessed and discussed since it was demonstrated that STING contributes to antitumor immunity as a spontaneous trigger of type I IFNs in the tumor microenvironment and a therapeutic target for enhancing cancer immunotherapy.[11–14] Although the main producer of STING-triggered type I IFNs in the tumor microenvironment still remains controversial, $CD11c^+$ dendritic cells, $CD11b^+$ myeloid cells, and endothelial cells are proposed as a sourse of type I IFNs via STING activation.[11,15,16] Based on these findings in several mouse models, STING ligands are regarded as one of the promising immune adjuvants for promoting antitumor immune responses.[17,18] Especially, intratumoral injections of CDNs effectively enhance production of type I IFNs and migration of $CD8^+$ T-cells, and thereby suppressing tumor growth although there may be some difficulties in direct injection into the tumor site.[17] Furthermore, we found that $CD11b^+Ly6C^{high}$ macrophages are recruited in the tumor site in a STING-dependent manner after intrartumoral injection of cGAMP and show inflammatory phenotypes, i.e. induction of TNF-α, type I IFNs, and T-cell-attracting chemokines. Because depletion of the macrophages

CONTACT Takayuki Ohkuri ohkurit@asahikawa-med.ac.jp; Hiroya Kobayashi hiroya@asahikawa-med.ac.jp Department of Pathology, Asahikawa Medical University, Midorigaoka-Higashi 2-1-1, Asahikawa, 078-8510, Japan.

[†] These authors contributed equally to this article.

© 2018 Takayuki Ohkuri, Akemi Kosaka, Toshihiro Nagato, and Hiroya Kobayashi. Published with license by Taylor & Francis
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

by using clodronate liposome abrogated the antitumor effect of STING activation, it is suggested that STING-triggered macrophages would contribute to antitumor immunity.[19] It, however, remains to be determined which signaling cascade is activated in the macrophages stimulated with STING agonists like cGAMP.

Alternatively activated and polarized macrophages in STAT6 signaling pathway

M2 macrophages are further classified into three forms based on the activation and polarization status, which are referred to as M2a, M2b, and M2c macrophages.[20] M2a macrophages induced by IL-4 or IL-13 are so-called "alternatively activated macrophages" and produce IL-10 and IL-1 receptor antagonist. M2b macrophages produce high amounts of IL-10 and low levels of IL-12 and are induced by combined immune complexes and TLR/IL-1R ligands. M2c macrophages are polarized by IL-10, glucocorticoid, or secosteroid hormones and characterized by high production of IL-10 and TGF-β with deactivated status.[21]

M2-polarized macrophages in the tumor site, referred to as tumor-associated macrophages (TAMs),[22,23] suppress antitumor activity of immune cells and accelerate tumor progression via producing TGF-β and IL-10 and promoting angiogenesis, respectively.[24] Stimulation with IL-4 and IL-13 induces M2 polarization via STAT6 activation and translocation, enhancing expression of several transcription factors such as peroxisome proliferator activated receptors (PPARs), PPARδ and PPARγ and PPARγ-coactivator-1β (PGC-1β). PPARδ is required for expression of arginase-1, which is one of the critical molecules in suppressing antitumor immunity[25] and is also reported to be induced by IL-6/STAT3 signaling cascade.[26] When arginine is starved in the tumor microenvironment by TAMs, NO production by M1 macrophages is reduced and MHC class II expression is down-regulated in antigen-presenting cells including macrophages, leading to suppress the immune responses to tumors.[26,27] In addition to increasing arginase-1 expression, PPARδ plays a role in maintaining expression of M2 macrophage-related molecules such as resistin-like α, mannose receptor, chitinase 3-like 3, and programmed cell death 1 ligand 2 in response to IL-4/STAT6 signals.[25] In contrast, PPARγ is involved in transcriptional regulation of lipolysis, fatty acid uptake, and β-oxidation of fatty acids, which are associated with maturation of alternatively activated status in macrophages.[28] Furthermore, PGC-1β enhances maturation of anti-inflammatory alternatively activated macrophages as a coactivator of STAT6.[29] STAT6 signaling cascade plays a critical role in polarizing macrophages into M2 phenotype via cooperating with the relevant transcription factors which STAT6 promoted itself. Thus, because STAT6 is phosphorylated by STING activation, STING ligands would also polarize macrophages into M2 phenotype as well as IL-4 and IL-13.

Conclusions: Effects of STING signaling on macrophage phenotype

STING activates both IRF3 and IRF7 and STAT6 for inducing type I IFNs and promoting inflammatory responses, respectively after receiving CDNs. It is therefore expected that STING-triggered macrophages show "classically" and "alternatively" activated status. This raises the question of how much STING signaling polarizes macrophages into M1 and/or M2 phenotypes. In our previous study, STING-triggered tumor-infiltrating macrophages showed more M1-like phenotype compared with TAMs.[19] However, TAMs are polarized M2 phenotype and a number of factors will be involved in the activation status of macrophages in the tumor microenvironment. Simply designed experiments *in vitro*, therefore, are required to answer the above question, helping us to understand how STING activation impacts on the macrophages in tumor-bearing host when STING ligands are used as an immune adjuvant in antitumor immunotherapy.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

1. Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature. 2008;455:674–78. doi:10.1038/nature07317. PMID:18724357.
2. Sun W, Li Y, Chen L, Chen H, You F, Zhou X, Zhou Y, Zhai Z, Chen D, Jiang Z. ERIS, an endoplasmic reticulum IFN stimulator, activates innate immune signaling through dimerization. Proc Natl Acad Sci U S A. 2009;106:8653–58. doi:10.1073/pnas.0900850106. PMID:19433799.
3. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol. 2000;164:6166–73. doi:10.4049/jimmunol.164.12.6166. PMID:10843666.
4. Van Ginderachter JA, Movahedi K, Hassanzadeh Ghassabeh G, Meerschaut S, Beschin A, Raes G, De Baetselier P. Classical and alternative activation of mononuclear phagocytes: picking the best of both worlds for tumor promotion. Immunobiology. 2006;211:487–501. doi:10.1016/j.imbio.2006.06.002. PMID:16920488.
5. Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003;3:23–35. doi:10.1038/nri978. PMID:12511873.
6. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity. 2014;41:14–20. doi:10.1016/j.immuni.2014.06.008. PMID:25035950.
7. Suschak JJ, Wang S, Fitzgerald KA, Lu S. A cGAS-Independent STING/IRF7 Pathway Mediates the Immunogenicity of DNA Vaccines. J Immunol. 2016;196:310–16. doi:10.4049/jimmunol.1501836. PMID:26590319.
8. Chen H, Sun H, You F, Sun W, Zhou X, Chen L, Yang J, Wang Y, Tang H, Guan Y, et al. Activation of STAT6 by STING is critical for antiviral innate immunity. Cell. 2011;147:436–46. doi:10.1016/j.cell.2011.09.022. PMID:22000020.
9. Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature. 2009;461:788–92. doi:10.1038/nature08476. PMID:19776740.
10. Prantner D, Darville T, Nagarajan UM. Stimulator of IFN gene is critical for induction of IFN-beta during Chlamydia muridarum infection. J Immunol. 2010;184:2551–60. doi:10.4049/jimmunol.0903704. PMID:20107183.
11. Ohkuri T, Ghosh A, Kosaka A, Zhu J, Ikeura M, David M, Watkins SC, Sarkar SN, Okada H. STING contributes to antiglioma immunity via triggering type I IFN signals in the tumor microenvironment. Cancer Immunol Res. 2014;2:1199–208. doi:10.1158/2326-6066.CIR-14-0099. PMID:25300859.
12. Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MY, Duggan R, Wang Y, Barber GN, Fitzgerald KA, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of

immunogenic tumors. Immunity. 2014;41:830–42. doi:10.1016/j.immuni.2014.10.017. PMID:25517615.
13. Gajewski TF. The Next Hurdle in Cancer Immunotherapy: Overcoming the Non-T-Cell-Inflamed Tumor Microenvironment. Semin Oncol. 2015;42:663–71. doi:10.1053/j.seminoncol.2015.05.011. PMID:26320069.
14. Corrales L, Matson V, Flood B, Spranger S, Gajewski TF. Innate immune signaling and regulation in cancer immunotherapy. Cell Res. 2017;27:96–108. doi:10.1038/cr.2016.149. PMID:27981969.
15. Klarquist J, Hennies CM, Lehn MA, Reboulet RA, Feau S, Janssen EM. STING-mediated DNA sensing promotes antitumor and autoimmune responses to dying cells. J Immunol. 2014;193:6124–34. doi:10.4049/jimmunol.1401869. PMID:25385820.
16. Demaria O, De Gassart A, Coso S, Gestermann N, Di Domizio J, Flatz L, Gaide O, Michielin O, Hwu P, Petrova TV, et al. STING activation of tumor endothelial cells initiates spontaneous and therapeutic antitumor immunity. Proc Natl Acad Sci U S A. 2015;112:15408–13. doi:10.1073/pnas.1512832112. PMID:26607445.
17. Chandra D, Quispe-Tintaya W, Jahangir A, Asafu-Adjei D, Ramos I, Sintim HO, Zhou J, Hayakawa Y, Karaolis DK, Gravekamp C. STING ligand c-di-GMP improves cancer vaccination against metastatic breast cancer. Cancer Immunol Res. 2014;2:901–10. doi:10.1158/2326-6066.CIR-13-0123. PMID:24913717.
18. Corrales L, Glickman LH, McWhirter SM, Kanne DB, Sivick KE, Katibah GE, Woo SR, Lemmens E, Banda T, Leong JJ, et al. Direct activation of sting in the tumor microenvironment leads to potent and systemic tumor regression and immunity. Cell Rep. 2015;11:1018–30. doi:10.1016/j.celrep.2015.04.031. PMID:25959818.
19. Ohkuri T, Kosaka A, Ishibashi K, Kumai T, Hirata Y, Ohara K, Nagato T, Oikawa K, Aoki N, Harabuchi Y, et al. Intratumoral administration of cGAMP transiently accumulates potent macrophages for anti-tumor immunity at a mouse tumor site. Cancer Immunol Immunother. 2017;66:705–16. doi:10.1007/s00262-017-1975-1. PMID:28243692.
20. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25:677–86. doi:10.1016/j.it.2004.09.015. PMID:15530839.
21. Goerdt S, Orfanos CE. Other functions, other genes: alternative activation of antigen-presenting cells. Immunity. 1999;10:137–42. doi:10.1016/S1074-7613(00)80014-X. PMID:10072066.
22. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357:539–45. doi:10.1016/S0140-6736(00)04046-0. PMID:11229684.
23. Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, Rimoldi M, Biswas SK, Allavena P, Mantovani A. Macrophage polarization in tumour progression. Semin Cancer Biol. 2008;18:349–55. doi:10.1016/j.semcancer.2008.03.004. PMID:18467122.
24. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol. 2009;27:451–83. doi:10.1146/annurev.immunol.021908.132532. PMID:19105661.
25. Odegaard JI, Ricardo-Gonzalez RR, Red Eagle A, Vats D, Morel CR, Goforth MH, Subramanian V, Mukundan L, Ferrante AW, Chawla A. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. Cell metabolism. 2008;7:496–507. doi:10.1016/j.cmet.2008.04.003. PMID:18522831.
26. Narita Y, Kitamura H, Wakita D, Sumida K, Masuko K, Terada S, Nakano K, Nishimura T. The key role of IL-6-arginase cascade for inducing dendritic cell-dependent CD4(+) T cell dysfunction in tumor-bearing mice. J Immunol. 2013;190:812–20. doi:10.4049/jimmunol.1103797. PMID:23248265.
27. Rutschman R, Lang R, Hesse M, Ihle JN, Wynn TA, Murray PJ. Cutting edge: Stat6-dependent substrate depletion regulates nitric oxide production. J Immunol. 2001;166:2173–77. doi:10.4049/jimmunol.166.4.2173. PMID:11160269.
28. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red Eagle A, Vats D, Brombacher F, Ferrante AW, Chawla A. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature. 2007;447:1116–20. doi:10.1038/nature05894. PMID:17515919.
29. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, Wagner RA, Greaves DR, Murray PJ, Chawla A. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell Metab. 2006;4:13–24. doi:10.1016/j.cmet.2006.05.011. PMID:16814729.