RESEARCH UPDATE (MINIREVIEW)
J Bacteriol Virol. Vol 49. No 4. December 2019; 49(4): 230-236
http://dx.doi.org/10.4167/jbv.2019.49.4.230
eISSN 2093-0249

JBV

PD-1: A Negative Regulator of Phagocytosis by Tumour-Associated Macrophages in Colon Cancer

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Received : May 10, 2019
Revised : October 15, 2019
Accepted : October 21, 2019

No potential conflict of interest relevant to this article was reported.

Programmed cell death protein 1 (PD-1) is an immuno-inhibitory cell surface receptor protein of the myeloid, and lymphoid cell. PD-L1 is the ligand of PD-1, which is abundant in different malignant tissue e.g. skin, colon and breast cancer. PD-1/PD-L1 interaction helps the tumour cell to escape from the immune response by limiting TCR mediated T lymphocytes proliferation. Recently, PD-1 or PD-L1 blocking immunotherapy proved their efficacy in the treatment of different cancers. However, PD-1/PD-L1 interaction is well studied in T lymphocytes, but little is known about its function in tumour-associated macrophages (TAMs). In the tumour microenvironment, phagocytosis by TAMs plays a vital role in the immune response. In this review, the significance of PD-1 expression by TAMs and how it influences tumour immunity will be discussed. Recently, it has been found that PD-1 can express by TAMs and its expression level is directly related to duration and stages of colon cancer. TAMs expression of PD-1 was shown to be related to significant depletion of cancer cell phagocytosis. Monoclonal antibody against either PD-1 or PD-L1 in mice model of colon cancer promotes tumour cell phagocytosis by TAMs, thereby limiting the growth of the tumour and increase life expectancy. Therefore, PD-1 can be a promising target in macrophage-mediated immune therapy.

Key Words: PD-1, Tumour-associated macrophages, Colon cancer

INTRODUCTION

Colorectal cancer is the third most common cancer worldwide and responsible for about 8.5% of all cancer-related death every year (1). There are several factors such as increasing age, obesity, high fat diet, alcohol intake, cigarette smoking, physical inactivity are related to colorectal cancer (2). A positive family history of colorectal cancer and pre-existing inflammatory bowel disease or adenomatous polyp also make a person prone to develop colorectal cancer (2). The complex pathogenesis of colorectal cancer begins with the formation of adenomatous polyp of sporadic origin with epithelial dysplasia of intestinal crypts (3). This dysplastic change of intestinal epithelium is the result of mutation of several oncogenes such as KRas, c-myc, β-catenin and loss of some tumour suppressor gene, especially APC (3, 4). Depletion of APC causes a rapid loss of various cellular constituent of intestinal epithelial cell and ultimately damage the defensive epithelial barrier (5). So intestinal microbiota gets access to invade colonic mucosa

and established an inflammatory microenvironment by producing IL17, IL23 like pro-inflammatory cytokines (5). So, inflammation has a significant contribution to the pathogenesis of colorectal cancer (3, 4). In this inflammatory microenvironment, both innate and adaptive immunity are equally responsible to produce an immune response (6). Immune response in tumour microenvironment depends on the types of immune cell that involved to develop response as well as their location and abundancy (6). Macrophages in the tumour microenvironment, well known as tumour associated macrophage (TAMs) are the most abundant innate immune cell (6). They can enhance tumour progression by promoting angiogenesis, tumour cell invasion, migration and intravasation (7). They also suppress antitumor immune responses through the expression of several different immune-inhibitory checkpoint receptor protein (7). Also, the abundance of TAMs in the tumour microenvironment is closely related to the fatal outcome of colorectal cancer in the experimental model (3). The Previous study proposed that tumour cell phagocytosis by TAMs had decreased because of interaction between SIRPα and CD-47 (8). SIRPα, a receptor protein of macrophages, is activated after binding with transmembrane protein CD-47 of tumour cells (8). This interaction initiates a cascade of signal transduction to inhibit phagocytosis of tumour cell by TAMs (8). Thus SIRPα/CD-47 interaction is considered as a major inhibitory check-point of the immune response in tumour immunity (8). Besides this, TAMs also express PD-1 receptor, which is also considered as an immune-suppressive checkpoint of tumour immunity (9). PD-1 is a receptor protein of the CD28 family and performs a vital role in the escape of tumour cells from an immune response (9). PD-1 consists of an extracellular region resembles the variable domain of immunoglobulin and a cytoplasmic region of a tyrosine-based immune-suppressive receptor motif (10). The extracellular part of PD-1 is structurally closely related to CTL-associated antigen 4 (CTLA-4), which performs a significant role in T lymphocytes homeostasis after binding with its ligand B7-1 and B7-2 (10). Because of this structural similarity, it has been hypothesized that ligand for PD-1 also belongs to the B7 protein family (10). Between two subtypes of this ligand, there is higher expression PD-L1 has been found in skin, lung, ovarian and several different human cancers (11). Also in squamous cell carcinoma, adenocarcinoma of colon and breast, PD-L1 overexpression is reported which helps tumour cells to escape lysis by the cytotoxic T cell (12). On the other hand, PD-L2 is highly expressed in different variety of B cell lymphoma (11). PD-1 when binds with its ligand PD-L1 in tumour cell can restrain T lymphocytes immune function through suppression of proliferation, survival and cytotoxic activity of effector T cell (13). This also promotes apoptosis of tumour-specific T cell and differentiation of $CD4^+$ T cells into $Foxp3^+$ regulatory T cells (9). Monoclonal antibodies against PD-1 has already shown efficacy in the treatment of melanoma, refractory Non-Hodgkin's lymphoma, renal cell carcinoma, colorectal cancer and non-small-cell lung cancer clinically (14). It is well studied that how the PD-1/PD-L1 pathway suppresses T lymphocyte activity but little is known about its role in TAMs. In gastric adenocarcinoma and NSCLC, TAMs infiltration is associated with the upregulated expression of PD-L1 in both tumour cells and immune cells (15, 16). A recent study reported that PD-1 can be expressed by tumour-associated macrophages in colon cancer to suppress tumour cells phagocytosis and can play a harmonious role along with SIRPα/CD-47 as a check-point of the immune response (17).

EXPRESSION OF PD-1 BY TUMOUR-ASSOCIATED MACROPHAGES IN COLON CANCER

Both PD-1 and its ligand has been overexpressed in some aggressive variety of solid tumours such as Breast, Colon and NSCL cancer in human and can suppress immunomodulatory T cell activation (18). Ultimately this PD-1 facilitate cancer progression and related to worse prognosis (19). PD-1/PD-L1 pathway also has a significant role in infection and autoimmunity (20). Increased expression of PD-1 on the surface of initially activated T lymphocytes can suppress the autoimmune response by limiting proliferation and cytokines production of self-reactive T cell (20). During chronic infections, there is a higher expression of PD-1 by T lymphocytes, which upon engaging with its ligand suppress proliferation of T cells, called exhausted T lymphocytes (20). Blockade of either PD-1 or its ligand helps T lymphocytes to regain their ability to proliferate and to perform cytotoxic functions via cytokines production (21). In sepsis, peritoneal macrophages also express PD-1 in a large number and contribute in pathogenesis of sepsis (22). But in the tumour microenvironment, PD-1 expression by macrophages was not well studied. To study PD-1 expression by macrophages Gordon, et al. introduced CT26 colon cancer cell in immunocompetent BALB/c mice (17). FACS analysis of tumour tissue after successful engraftment had shown,

around 50% of tumour-associated macrophages were PD-1$^+$. But PD-1 expression was insignificant in blood monocytes and other tissue macrophages (e.g. spleen). To confirm this high PD-1 expression, immunofluorescence assay of FACS sorted TAM had revealed a large number of cells expressed both PD-1 and macrophage marker CD68. Also in human colorectal cancer tissue, the number of PD-1$^+$ TAMs was much higher compared to PD-1$^-$ TAMs. Moreover, PD-1 expression by TAMs was not static. In mice model, PD-1 showed time and tumour size-dependent augmentation in its expression. Also in samples of human colon cancer, PD-1 expression by TAMs was related to stages of disease (17).

PD-1$^+$ TUMOUR-ASSOCIATED MACROPHAGES ARE MOSTLY PRO-TUMOUR M2 SUBTYPE

Based on tissue microenvironment macrophages can differentiate into the distinct subtype with distinct function (23). M1 (pro-inflammatory) macrophages can enhance immune response but M2 (anti-inflammatory or pro-tumour) subtype suppressed inflammatory response by inhibiting the function of effector T cell (23). FACS analysis of TAMs, obtained from CT26 tumour of mice demonstrated that typical markers of TAMs, CD11b and F4/80 was equal in both PD-1$^+$ cell and their counterpart. But PD-1$^+$ TAMs were mostly M2 type, as they demonstrate M2 macrophage marker profile (more CD206, CD11c and less MHC II). On the other hand PD-1$^-$ TAMs had an aptitude to express M1 marker profile (low CD206 and high MHC II). Also in the human sample of colon cancer, a large proportion of PD-1$^+$ TAMs was M2 or pro-tumour type (17). It is evident from the previous study that M2 macrophages influence tumour growth, invasion and metastasis significantly and can make worse prognosis (23). M2 macrophages can produce matrix metalloproteinases (MMP), that can breakdown the endothelial basement membrane and facilitate tumour cell invasion and metastasis (24). M2 cells also promote angiogenesis by producing vascular endothelial growth factors (25).

CD4 is a well-known T cell marker but also found on the myeloid cell e.g. in macrophages (26). Tumour-associated macrophages can also express CD4 on their surface which was confirmed by CD68 and CD4 co-expressing TAMs on immunofluorescence staining. Also, CD4 expression is significantly higher in PD-1$^+$ TAMs in both mice and human colon cancer tissue compared to its counterpart (17).

ORIGIN OF PD-1$^+$ TAMs AND THEIR MORPHOLOGY

Being a part of the mononuclear phagocytic system, macrophage was previously considered as a sole derivative of circulating blood monocytes (27). But some recent studies have challenged this concept with a proposal that macrophages arise from its embryonic progenitors of yolk sac in homeostatic condition (28). Studies come with the hypothesis that macrophages have local self-renewal capacity without contribution from blood monocytes (29). But in the intestine, macrophages are solely derived from blood monocytes because of its continual exposure to external stimuli (30). Also when there is infection or tissue damage, classical monocytes can readily involve in developing macrophages in all tissues (30). To determine the predecessor of TAMs in the inflammatory microenvironment of colon cancer, bone marrow transplantation model had been used. RFP$^+$ C57BL/6 mice (donor) bone-marrow were transplanted into GFP$^+$ C57BL/6 mice (host) after irradiation and later successful engraftment were confirmed by chimaerism of the donor myeloid cell, lymphoid cells and granulocyte in host blood. Then colon cancer cell, MC38 was introduced into the host mice and FACS analysis of tissue from established colon cancer revealed that a large proportion of PD-1$^+$ TAMs originated from RFP$^+$ donor bone marrow, not from tissue- habitant immune cells of host (17). So, they hypothesized that bone-marrow-derived macrophages are liable for the time-dependent increment of PD-1$^+$ TAMs in the cancer microenvironment, not the tissue-resident macrophages (17). PD-1$^+$ TAMs are morphologically different from PD-1$^-$ TAMs. On Giemsa staining, PD-1$^+$TAMs look large and foamy due to the presence of lysosome and phagosome filled with enormous, uncleaned phagocytic materials (17).

PD-1$^+$ TAMs INHIBIT PHAGOCYTOSIS OF TUMOUR CELL

Being a receptor protein of different immune cell, PD-1 has an exclusive role in immune escape of tumour cell (17). Some studies demonstrated that PD-1/PD-L1 signaling can suppress TCR-mediated proliferation of T cells and can downregulate co-stimulatory CD3/CD28 signal. Also, TCR and CD28 mediated signal strength if strong, it can inhibit the functional consequences of the PD-1/PD-L1 interaction (10). Moreover, PD-1 expression by TAMs can influence phagocytosis of tumour cell confirmed by both in vitro and in vivo study (17). An ex vivo phagocytosis assay of TAMs obtained from same CT26 tumour model that we discussed earlier, revealed that phagocytosis of GFP$^+$ Staphylococcus aureus bioparticles by PD-1 expressing TAMs was much less in contrast to its counterpart (17). In vivo study, BALB/c *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ immunocompromised mice had been used, which can express PD-1 on TAMs as like immunocompetent mice. They generated two subsets of CT26 cell having YFP, one with overexpressed PD-L1 to agonize PD-1 signaling and the other with knockout PD-L1 and engrafted into mice. After three weeks total phagocytosis level was evaluated by the percentage of YFP$^+$ TAMs. In PD-L1 overexpression group, PD-1 expressing TAMs had less phagocytic potency compared to PD-1$^-$ TAMs phagocytosis and inversely related to total phagocytosis level. But upon removal of PD-L1 (PD-L1 knockout group), PD-1$^+$ TAMs had significant improvement in phagocytic potency, where there was no change in PD-1$^-$ TAMs phagocytic ability. So, removal of PD-L1 can surpass the inhibitory impact of PD-1 on phagocytosis by TAMs. Moreover, tumour size was relatively smaller in PD-L1 knockout group compared to the opposite group because of improved phagocytosis by TAMs (17).

IMPROVED PHAGOCYTIC POTENCY OF TAMs UPON PD-1 AXIS BLOCKADE

PD-L1 or PD-1 (PD) blockade is a highly potential therapeutic strategy and has provided sustainable anti-tumour effect and prevent recurrence of different malignancy for a long time in several clinical trials (14). Among them, melanoma, lymphoma and bladder cancer were most responsive to PD-1 pathway occlusion therapy (14). This is not only because of overcoming T cell-mediated immune suppression (14) but also because of restoring the phagocytic potency of TAMs (17). To inhibit the PD-1/PD-L1 interaction, they introduced either antibody to mouse PD-1 or HAC (a tiny protein with PD-L1 blocking property) in DLD-1 xenograft NSG mice having PD-1$^+$ TAMs. To abstain any anti-tumour effect by the interaction between the cancer cell of mouse and antibody to mouse PD-1, here they engrafted human colon cancer cell DLD-1. DLD-1 cell can express both PD-L1 and GFP-luciferase. Three weeks later, removed tumour size was significantly small in the experimental group compared to control, because of improved phagocytosis by TAMs. Only TAMs PD-1/PD-L1 axis was targeted by these inhibitors (anti-PD-1 antibody or HAC) as TAMs depletion with anti-CSF1R treatment abolished the effect of inhibitors. Removal of PD-L1 can reduce tumour size by promoting phagocytic potency of PD-1$^+$ TAMs (17). This suggests that the PD-1/PD-L1 axis is an antagonist of TAMs phagocytic efficacy (17). Therefore, tumour immunity can be intensified with the suppression of either PD-1 or its ligand in TAMs (17). Also, PD pathway blockade therapy has less Immune-related toxicities compared to other immune blockade strategies such as cytotoxic T lymphocyte antigen-4 (CTLA-4) occlusion therapy. So, PD-1 blockade therapy considered as a potential treatment option in cancer immune therapy (14).

INTERACTION BETWEEN PRE-EXISTING ANTI-CD47 THERAPY AND PD-1 AXIS BLOCKADE THERAPY

SIRP-α/CD-47 is considered as a principle immune regulatory checkpoint on macrophage in tumour immunity and CD-47 targeted therapy is in the clinical trial (8). Moreover, PD-1/PD-L1 targeted therapy can potentiate the efficacy of pre-existing anti-CD-47 therapy (17). To demonstrate this, DLD xenograft NSG mice were treated with PBS (control), anti-CD-47, HAC, both HAC and anti-CD-47 in four separate groups. Though HAC and anti-CD47 single therapy individually can suppress tumour growth, combined therapy was more effective than monotherapy in reducing tumour size and increasing survival of mice model (17).

CLOSING REMARKS

In conclusion, PD-1 is not only an immune-inhibitory receptor of T cell but also in tumour- associated macrophages its expre ssion is high and increases with disease stage. PD-1^{+} TAM has a distinct surface marker profile, morphology and less phagoc ytic potency compared to its counterpart. Also, inhibition of PD-1/PD-L1 interaction can enhance phagocytosis by PD-1^{+} TA Ms. Understanding the role of PD-1 expressed by TAMs may facilitate to develop a new therapeutic target in cancer immun e therapy.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. NRF-2019R1A2C1008275).

REFERENCES

1) Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:359–86.

2) Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg 2009;22:191–7.

3) Huang YJ, Yang CK, Wei PL, Huynh TT, Whang-Peng J, Meng TC, *et al.* Ovatodiolide suppresses colon tumorigenesis and prevents polarization of M2 tumor-associated macrophages through YAP oncogenic pathways. J Hematol Oncol 2017; 10:60.

4) Sanchez-Lopez E, Flashner-Abramson E, Shalapour S, Zhong Z, Taniguchi K, Levitzki A, *et al.* Targeting colorectal cancer via its microenvironment by inhibiting IGF-1 receptor-insulin receptor substrate and STAT3 signaling. Oncogene 2016; 35:2634–44.

5) Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, *et al.* Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 2012;491:254–8.

6) Waniczek D, Lorenc Z, Śnietura M, Wesecki M, Kopec A, Muc-Wierzgoń M. Tumor-associated macrophages and regulatory T cells infiltration and the clinical outcome in colorectal cancer. Arch Immunol Ther Exp (Warsz) 2017;65: 445-54.

7) Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 2006; 124:263–6.

8) Chao MP, Alizadeh AA, Tang C, Myklebust JH, Varghese B, Gill S, *et al.* Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. Cell 2010;142:699–713.

9) Zitvogel L, Kroemer G. Targeting PD-1/PD-L1 interactions for cancer immunotherapy. Oncoimmunology 2012;1:1223–5.

10) Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, *et al.* Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000;192:

1027-34.

11) Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64.

12) Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A 2002;99:12293–7.

13) Tseng SY, Otsuji M, Gorski K, Huang X, Slansky JE, Pai SI, *et al.* B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. J Exp Med 2001;193:839–46.

14) Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. Sci Transl Med 2016;8:328rv4.

15) Harada K, Dong X, Estrella JS, Correa AM, Xu Y, Hofstetter WL, *et al.* Tumor-associated macrophage infiltration is highly associated with PD-L1 expression in gastric adenocarcinoma. Gastric Cancer 2018;21:31-40.

16) Sumitomo R, Hirai T, Fujita M, Murakami H, Otake Y, Huang CL. PD-L1 expression on tumor-infiltrating immune cells is highly associated with M2 TAM and aggressive malignant potential in patients with resected non-small cell lung cancer. Lung Cancer 2019;136:136-44.

17) Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, *et al.* PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature 2017;545:495-9.

18) Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, *et al.* Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. Cancer Epidemiol Biomarkers Prev 2014;23:2965-70.

19) Wu P, Wu D, Li L, Chai Y, Huang J. PD-L1 and survival in solid tumors: a meta-analysis. PLoS One 2015;10:e0131403.

20) Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nature Immunol 2007;8:239–45.

21) Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, *et al.* Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;439:682–7.

22) Huang X, Venet F, Wang YL, Lepape A, Yuan Z, Chen Y, *et al.* PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. Proc Natl Acad Sci U S A 2009;106: 6303–8.

23) Yuan R, Li S, Geng H, Wang X, Guan Q, Li X, *et al.* Reversing the polarization of tumor-associated macrophages inhibits tumor metastasis. Int Immunopharmacol 2017;49:30-7.

24) Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 2010;141: 52-67.

25) Rouhi P, Lee SL, Cao Z, Hedlund EM, Jensen LD, Cao Y. Pathological angiogenesis facilitates tumor cell dissemination and metastasis. Cell Cycle 2010;9:913-7.

26) Zhen A, Krutzik SR, Levin BR, Kasparian S, Zack JA, Kitchen SG. CD4 ligation on human blood monocytes triggers macrophage differentiation and enhances HIV infection. J Virol 2014;88:9934–46.

27) Van Furth R, Chon ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Bull World Health Organ 1972;46:845–52.

28) Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, *et al.* A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 2012;336:86–90.

29) Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci 2007;10:1538–43.

30) Bain CC, Bravo-Blas A, Scott CL, Perdiguero EG, Geissmann F, Henri S, *et al.* Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. Nat Immunol 2014;15:929–37.