AN UPDATE REVIEW ON IMMUNOSUPPRESSIVE CELLS; MYELOID DERIVED SUPPRESSOR CELLS (MDSCs) IN CANCERS

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Abstract
Myeloid derived suppressor cells (MDSCs) are heterogeneous subsets of immune cells and they function to inhibit host T cells activation leading to tumour growth. Currently, the majority of studies support key contributions of MDSCs to tumour progression via direct mechanisms immune mediated and indirect mechanism which is not directly associated with immune suppression. Due to the complexity of MDSCs heterogeneity, the aspect of MDSCs phenotype, morphology and function is poorly investigated up to date. And for this reason, this review will provide a comprehensive understanding of the role and function of MDSCs in cancer patients. Targeting the immunosuppressive cells MDSCs may improve the efficacy of immunotherapy in cancer patients in future.

Keywords: Myeloid derived suppressor cells (MDSCs), macrophages, dendritic cells, natural killer cells, cancer, immunotherapy, vaccines
Abbreviations

Antigen presenting cells: APCs
Arginase 1: ARG1
Bacillus Calmette–Guérin: BCG
Colorectal cancer: CRC
Cyclooxygenase 2: COX-2
Dendritic cells: DCs
Granulocytic Myeloid-derived suppressive cells: G-MDSCs
Interleukin-2: IL-2
Interferon-alpha: IFN-α
Interferon-gamma: IFN-γ
Inducible nitric oxide: iNOS
Myeloid-derived suppressive cells: MDSCs
Monocytic Myeloid-derived suppressive cells: M-MDSCs
Natural killer cells: NK
Nuclear factor kappa β: NF-κβ
Reactive oxygen species: ROS
Prostaglandin E2: PGE2
World Health Organisation: WHO
Tumour infiltrating lymphocytes: TILs
T regulatory cells: Treg cells
Tumour-associated antigens: TAA
Transforming growth factor beta: TGF-β
T regulatory cells: Treg
Tumour microenvironment: TME
Signal transducer and activator of transcription: STAT
1. Introduction

Cancer is the second leading cause of deaths worldwide with 9.6 million deaths in 2018, it is estimated about 1 in 6 deaths of cancer patients, according to the latest report from the WHO. Cancer treatment by surgery, radiotherapy and chemotherapy, alone or in combination with other drugs, have been effective at treating the primary stage of the cancer but with severe side effects as failed to control and reduce cancer spread (metastasis) (Couzin-Frankel, 2013). Moreover, new cancer therapy became urgently needed and scientists currently directed the focus of research into understanding the mechanism of the tumourgenesis and the mechanism of immune system.

To understand the immune system, the immune system works basically by distinguishing self from non-self-antigens, and it has the ability to detect and eliminate any foreign antigens. The immune system continuously failed to identify, destroy and eradicate the transformed cells during tumour progression in cancer patients, however, tumour infiltrating lymphocytes (TILs) (de Lima et al., 2020) and antigen presenting cells (APCs) are shown to be non-functional within tumour tissue samples, decreased number of mature dendritic cells (DCs) in the peripheral blood of cancer patients for further information please refer to review (Kumar, Patel, Tcyganov, & Gabrilovich, 2016; Marvel & Gabrilovich, 2015). A study reported that the antigen-specific CD4+ T cells were rendered unresponsive by the tumours growth, suggesting that the T cells function can also be inhibited by cancer cells (Cuenca et al., 2003).

Tumours can induce immunosuppression by the release of microvesicles or exosomes (which are found in the body fluids of cancer patients of melanoma and colorectal cancer (CRC)). The release of microvesicles can promote monocytes and myeloid derived-suppressive cells (MDSCs) differentiations and thus support tumours escape the immune system recognition (Valenti et al., 2007).

However, a new treatment for cancer known as (immunotherapy) has been developed in recent years. Immunotherapy works to boost the immune system by enhancing the immune surveillance, destroy abnormal
cells and ultimately improve the survival rate of cancer patients (Sun et al., 2019). In contrast to immunotherapy, cancer vaccines are also considered as the latest application of active immunotherapy in cancer prevention or may cancer treatment (Aly, 2012; Rosenberg, Yang, & Restifo, 2004). Cancer vaccine is to degrade and process tumour antigens by APCs and then sample it via self-antigens known as major histocompatibility complex (MHC) class one (I) and/or class two (II) to T cells which then induce an endogenous, long-lasting tumour antigen-specific immune response by T cells (Couzin-Frankel, 2013; Schuster, Nechansky, & Kircheis, 2006). The efficacy of vaccines are usually boosted with the combination of adjuvant such as Bacillus Calmette–Guérin (BCG), interleukin two or twelve (IL-2 or IL-12) in order to maintain cellular as well as humoral immune responses (Habal et al., 2001; Villinger, 2003).

On the other hand, immunotherapy has been successfully used in clinical trials to treat a variety of tumours such of these immunotherapeutic treatment cytokines, IL-2 and interferon alpha (IFN-α), which were clinically approved and used for treatment of Kaposi’s sarcoma and leukaemia (Hurley & Chapman, 2005; J. C. Yang et al., 2003). In addition to that, monoclonal antibodies (mAb) based immunotherapy such as Rituximab, Cetuximab and Trastuzumab were tested for the treatment of leukaemia, non-Hodgkin’s lymphoma, breast and CRC (Couzin-Frankel, 2013; Schuster et al., 2006).

Furthermore, adoptive cell therapy (ACT – using autologous TILs) has been used as the most effective immunotherapy treatment for metastatic patients, particularly melanoma, and showed approximately 50% of tumour regression in cancer patients. The successful use of ACT in melanoma patients has opened new opportunities to patients with different cancer types and is a promising as new approach to cancer treatment (Rosenberg, Restifo, Yang, Morgan, & Dudley, 2008).

Despite these developments strategy in immunotherapy, the potential effectiveness of cancer immunotherapy has been minimised by the immune system particularly by the function of T regulatory cells (Tregs) and MDSCs. MDSCs are phenotypically and functionally heterogeneous subsets of immune cells (as discussed in subsequent sections) and they function to inhibit host T cell activity against tumour associated-antigens (TAA) and as a result this will a) minimise the action of anticancer immunotherapeutic approaches, b) inhibiting T cells
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activation and c) leading to tumour growth (Kumar, Patel, et al., 2016; Nagaraj et al., 2007). Targeting the immunosuppressive cells; Tregs and MDSCs may improve the efficacy of immunotherapy in cancer patients. Taken together, the aspect of MDSCs is poorly investigated and for this reason this review will focus on MDSCs and provide a comprehensive understanding of their roles in cancer patients.

2. Definition of MDSCs

MDSC cells were first described in association with cancer development and progression for more than thirty years (Ostrand-Rosenberg & Sinha, 2009). MDSCs represent the majority of immature and haematopoietic immune cells but with various cell subsets, physiological, functional and pathological roles (Damuzzo et al., 2015; Gabrilovich, Ostrand-Rosenberg, & Bronte, 2012; Khaled, Ammori, & Elkord, 2013). Immature myeloid cells are produced in the bone marrow from common myeloid progenitor cells and differentiate in order to become mature cells into the three functional myeloid subsets; macrophages (MΦ), dendritic cells (DCs) and granulocytes (with no systemic immunosuppressive effects) in normal individuals (Gabrilovich, 2017). During pathological conditions, immature myeloid cells are also produced but they functionally differ in comparisons to that produced in normal conditions, with an expansion in their immunosuppressive activity such as production reactive oxygen species (ROS), nitric oxide (NO), and anti-inflammatory cytokines (Condamine, Mastio, & Gabrilovich, 2015; Youn, Collazo, Shalova, Biswas, & Gabrilovich, 2012). Studies reported that the accumulation of MDSCs implicated in inflammation and tumours (Bunt, Sinha, Clements, Leips, & Ostrand-Rosenberg, 2006; Capietto et al., 2013; Ostrand-Rosenberg & Sinha, 2009; Porembka et al., 2012).

2.1. MDSCs characterisation in Mice and Humans

MDSCs in mice and humans are defined as a mixture of activated immature myeloid cells; granulocytic or polymorphonuclear (PMN) and monocytic or mononuclear cells, which can be identified by the expression of cell surface markers (Damuzzo et al., 2015) as discussed in more details in subsequent sections.

2.2. MDSCs in Mice
MDSCs in mice are identified by the co-expression of the myeloid differentiation antigen (Gr-1) and cluster differentiation (CD11b) (Youn et al., 2012). Gr-1 consists of two epitopes: Ly-6G and Ly-6C, the level of Ly-6C epitopes distinguish the granulocytic (G-MDSCs) and monocytic (M-MDSCs) phenotypic. MDSCs express CD11b Ly-6G and Ly-6C, in particular, G-MDSCs cells express CD11b^ Ly-6G^ and Ly-6C^low^, whereas M-MDSCs express CD11b^ Ly-6G^ and Ly-6C^high^ (Movahedi et al., 2008; Movahedi et al., 2010; R. Yang et al., 2006; Youn et al., 2012). The phenotypical features of the G-MDSCs and M-MDSCs subsets are most often are associated with population of immature myeloid cells, share structural, but they differ in functional activity and their exact roles in pathological conditions remain elusive. However, several reports indicated that they implicated in cancer and autoimmune diseases (Cripps & Gorham, 2011; Greten, Manns, & Korangy, 2011; Nagaraj et al., 2009; Zhao, Wu, Shao, Shi, & Zhao, 2015). In tumour models, it was shown that G- and M-MDSC subpopulations were expanded with greater expansion of the G-MDSC population than M-MDSC population (Gabrilovich & Nagaraj, 2009; Zhou et al., 2010). In most tumour-bearing mice, the G-MDSC subsets increase in parallel with the increase of M-MDSCs population in limited tumour models (Youn, Nagaraj, Collazo, & Gabrilovich, 2008). Other findings reported that the expansion of MDSCs is not associated with their suppressive role but it is linked to their function activity within the tumour environment (Gabrilovich, Ciernik, & Carbone, 1996). A study reported that STAT3 inhibition in tumour-bearing mice led to MDSC depletion in spleens but not in tumours (Kumar, Cheng, et al., 2016). Scientists attempted to characterise the nature of the G- and M-MDSCs in mice tumour model, and studies demonstrated that the phenotype and cell markers of G-MDSCs are relatively similar to normal mature neutrophils, but their functional activity are different (Damuzzo et al., 2015; Fridlender et al., 2012; Youn et al., 2012).

2.3. MDSCs in Human

The phenotype of human MDSCs is defined as suppressive monocyte that contains membrane cell marker CD14 (Mielcarek, Martin, & Torok-Storb, 1997; J. Talmadge et al., 1996). Later on, human MDSCs from head and neck cancer patients were described to co-express CD34 and these MDSCs were defined as haematopoietic progenitor cells as they were negative for the expression CD3, CD19, CD56 and CD13. Further
investigations were performed and identified the phenotypic expression of human MDSCs as characterised with the absence of human leukocyte antigen D-related (HLA-DR) expression and one or both myeloid marker (CD33 or CD11b) and reviewed (J. E. Talmadge & Gabrilovich, 2013).

Given the history of MDSCs, human MDSCs in cancer patients were initially defined as CD11b⁺, CD33⁺, CD14⁻ and HLA-DR⁻ (J. E. Talmadge & Gabrilovich, 2013). Because of the heterogeneity of MDSCs subset, the identification of human G- and M-MDSCs population have been challenging and thus were examined in different types of human cancers (Lechner et al., 2011; Nagaraj et al., 2010). For example, studies in renal cell carcinoma (RCC) found that G-MDSCs express CD15, whilst M-MDSCs subsets were positive for CD14 expression (Ko et al., 2009; Poschke, Mougakakos, Hansson, Masucci, & Kiessling, 2010; Rodriguez et al., 2009).

Taken together, this suggests that the MDSCs subsets may express several surface molecules that assist in differentiation, and immunosuppressive activity function of MDSCs. Further investigations should be performed to understand the role, function and the characteristics cell surface markers of MDSCs subsets in the tumour microenvironment (TME).

2.4. MDSC Expansion and activation in cancer

The majority of studies from tumour bearing mice and human cancers reported that the induction and expansion of MDSCs in cancer environment is mediated by multiple factors including cytokines, growth factors and pro-inflammatory molecules (Serafini, Borrello, & Bronte, 2006; Umansky, Blattner, Gebhardt, & Utikal, 2016). The potential implicated factors in human cancers are divided into two groups: the first group: MDSC expansion promoting factors and the second group MDSC activating factors (Gabrilovich & Nagaraj, 2009). The MDSCs expansion is facilitated by triggering a signalling cascades that regulate proliferation, differentiation, cell survival and cell death via Janus tyrosine kinase (JAK) protein family members and signal transducer and activator of transcription 3 (STAT3) (Gabrilovich & Nagaraj, 2009; Zhang et al., 2010). In particular, a study showed that STAT3 is the main transcription factor involved in MDSCs expansion. Elevated levels of vascular
endothelial growth factor (VEGF) were associated with down-regulation of the activated STAT3 and ROS production in MDSC (Jayaraman et al., 2012). These evidence indicated that the mechanism that involved via the modulation of tumour VEGF secretion and upregulation of STAT3 and ROS in MDSC (Qu, Yan, Blum, Kapur, & Du, 2011; Wu, Du, Li, Qu, & Yan, 2011). A study demonstrated that in ovarian cnacer VEGF expression induced MDSCs leading to the inhibition of local immunity and poor cancer prognosis (Horikawa et al., 2017).

However, it was reported that downstream of STAT3 may regulate MDSCs expansion by S100A8 and S100A9 proteins induction (Cheng et al., 2008). The mentioned proteins are calcium-binding proteins that are released in response to cell damage and have been shown involved in the production of ROS and accumulation MDSCs, particularly G-MDSCs (Dufait et al., 2016; Turovskaya et al., 2008). In addition, it reported that activating factors also plays a critical role on MDSC expansion through multiple signalling pathways including STAT6, STAT1, and nuclear factor-κB (NF-κB) (Gabrilovich & Nagaraj, 2009). STAT1 is the major transcription factor that activated by IFN-γ. STAT1 is mainly responsible for upregulation of Arginase (ARG1) and inducible nitric oxide synthase (iNOS) in MDSCs within the tumour microenvironment (Gabrilovich & Nagaraj, 2009).

2.5. Mechanisms of MDSC in cancer:

The impact of MDSCs on cancer induction, expansion and including tumour angiogenesis and metastasis (Dysthe & Parihar, 2020), which is better described as two staged effects; the first stage is involved an abnormal myelopoiesis and recruitment of MDSCs into the tumour tissue. The second stage is an active MDSC cytokine production within the environment resulting in progression of cancer (Gabrilovich et al., 2012).

MDSCs mediate their tumour induced immunosuppression via a variety of potential mechanisms (Wang, Ding, Guo, & Wang, 2019). MDSCs mediate their T cells suppression in cancer through mainly two ways a) the direct cell contact with immune cells and b) through the effects of soluble mediators such as ARG1, iNOS, ROS, cyclooxygenase 2 (COX-2), prostaglandin E2 (PGE2), transforming growth factor beta (TGF-β), Treg cells and IL-10 (Bogdan, 2010; Condamine & Gabrilovich, 2011; Elliott, Doherty, Sheahan, & Ryan, 2017; Srivastava, Sinha, Clements,
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Rodriguez, & Ostrand-Rosenberg, 2010). The mechanism of action of these mediators are presented briefly in the following sections.

2.5.1 ARG1, iNOS and ROS

L-arginine metabolism has been recognised as a major key player in the suppressive activity. ARG1 and iNOS are expressed at high level in MDSCs and they utilise the L-arginine to produce urea and nitrogen (NO), and they are associated with an inhibition of T cells proliferation (by decreasing T cell receptors expression) (Bogdan, 2010; Raber et al., 2014; Rodriguez et al., 2009). Moreover, it has been shown that MDSCs inhibit T cells activation by degrading amino acid (cysteins) that is essential for T cells activation (Srivastava et al., 2010).

On the other hand, ROS production has been reported to be a major regulator of the suppressive activity of the G-MDSCs in both mice and human cancer (Qu, Boelte, & Lin, 2012).

2.5.2 PGE2 and COX-2

PGE2 and COX-2 are both potent mediators produced during inflammatory conditions and by many tumour cells as well. PGE2 were the main receptors found in human MDSCs (Fujita et al., 2011; Rodriguez et al., 2005). PGE2 functions to upregulate ARG1 and regulate MDSC and recruitment of T cell immune suppression (Fujita et al., 2011; Obermajer & Kalinski, 2012a, 2012b; Obermajer et al., 2012; Sinha, Clements, Fulton, & Ostrand-Rosenberg, 2007). A study demonstrated that PGE2-regulated accumulation of MDSCs and this contributes to the recovery of NK cells activity in vitro (Mao et al., 2014). In contrast to PGE2, COX-2 was over expressed in a number of human tumours including lung, colon, breast and prostate cancers (Nagaraj et al., 2010).

2.5.3 TGF-β

TGF-β is a multifunctional cytokine that associated with MDSCs and tumour growth, reviewed largely by (Letterio & Roberts, 1998). It has been demonstrated that patients with squamous cell carcinoma of the head and neck (SCCHN), their MDSC subset (CD14+HLA-DR- MDSC) were high numbers and produced higher levels of TGF-β than other MDSC subsets (Chikamatsu et al., 2012). Other studies demonstrated that the addition of monoclonal antibody (mAb) anti-TGF-β in combination
with anti-CD86 and anti-PD-L1 lead to T-cell proliferation and the secretion of IFN-γ. These evidence likely indicate that MDSC is able to produce TGF-β which likely has immunosuppressive effects as well as promotes tumour growth (Lu et al., 2011; Park & Lee, 2017).

2.5.4 Treg cells

The available data indicate that MDSCs are involved in Treg cells differentiation in various pathways. Huang and colleagues reported that the induction of Treg cells by MDSCs was IFN-γ dependent in the presence of IL-10 and antigen-associated activation of tumour-specific T cells (Huang et al., 2006). In mice model, a study demonstrated that tumour-infiltrating MDSCs express high levels of chemokines receptors CCR5 and cognate ligands CCL3, CCL4, and CCL5. When tumour tissue of mice were injected with CCL4 and CCL5, tumour-infiltrating Treg cells were increased (Lee et al., 2016; Schlecker et al., 2012).

Moreover, another study showed that CCL5 was preferentially expressed on CD4+ Foxp3+ Tregs in human pancreatic cancer and MDSCs recruit Treg cells into the tumour microenvironment in murine model of pancreatic cancer (Tan et al., 2009). In a mouse model of lymphoma, it was found that MDSCs induced Treg cells expansion through ARG1 pathway and presentation of tumour associated antigens (Bronte & Zanovello, 2005; Park & Lee, 2017). Therefore, further investigation is mandatory fill the gap of knowledge regarding the cellular interactions between Treg cells and MDSCs in cancer.

3. Conclusion

MDSC have been reported to be a key player in tumour associated immune evasion and it is characterised by a heterogeneous population of immature cells. Their accumulation has been reported and associated with the development of some human cancers, while their elimination have proven to enhance cancer immunotherapy in human cancers. However, there remain many questions to be answered, MDSCs phenotypic characterisation in different human cancers? What the exact mechanism of MDSCs in human cancers? How MDSCs mediate an antigen-specific T cell suppression? All these questions needs further investigations. This of course will help in future clinical trials which may improve immune-based therapeutic strategies modulation of MDSCs in cancers.
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مراجعه حديثه على الخلايا المثبطة للمناعة: الخلايا المثبطة (والمشتقة من النخاع) في السرطانات

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الملخص
إن الخلايا المثبطة للمناعة (المشتقة من النخاع) هي عبارة عن مجموعات فرعية غير متجانسة من الخلايا المناعية وتعمل على تثبيط تنشيط الخلايا التائية للعائل مما يؤدي إلى نمو السرطان. حاليا، معظم الدراسات تدعم الدور الرئيسي الذي تلعبه الخلايا المثبطة للمناعة لنمو السرطان عن طريق الاليات المباشرة بواسطة الجهاز المناعي والاليات الغير مباشرة التي لا تتطلب مباشرة تثبيط الجهاز المناعي. نظرا لتعقيد التركيب الغير متجانسة للخلايا المثبطة للمناعة فإن النمط الظاهري والشكلي والوظيفي لهذه الخلايا يفتقر للدراسة حتى الآن. ولهذا السبب، هذه الدراسة تتوفر مراجعه وفهما شاملا لدور ووظيفة الخلايا المثبطة للمناعة المشتقة من النخاع في مرضى السرطان. قد يؤدي فهم واستهداف الخلايا المثبطة للمناعة إلى تحسين فعالية العلاج المناعي في مرضى السرطان في المستقبل.