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Myeloid-derived-suppressor cells as regulators of the immune system

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Abstract

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells that expands during cancer, inflammation and infection, and that has a remarkable ability to suppress T-cell responses. These cells constitute a unique component of the immune system that regulates immune responses in healthy individuals and in the context of various diseases. In this Review, we discuss the origin, mechanisms of expansion and suppressive functions of MDSCs, as well as the potential to target these cells for therapeutic benefit.

Introduction

The first observations of suppressive myeloid cells were described more than 20 years ago in patients with cancer[1-3]. However, the functional importance of these cells in the immune system has only recently been appreciated due to accumulating evidence that has demonstrated their contribution to the negative regulation of immune responses during cancer and other diseases. It is now becoming increasingly clear that this activity is contained within a population known as myeloid-derived suppressor cells (MDSCs). Features common to all MDSCs are their myeloid origin, immature state and a remarkable ability to suppress T-cell responses (Box 1). In addition to their suppressive effects on adaptive immune responses, MDSCs have also been reported to regulate innate immune responses by modulating the cytokine production of macrophages[4]. Non-immunological functions of MDSC have also been described, such as the promotion of tumour angiogenesis, tumour-cell invasion and metastasis. However, as a discussion of these aspects of MDSC biology is beyond the scope of this article, the reader is referred to another recent Review on this topic[5].

MDSCs represent an intrinsic part of the myeloid-cell lineage and are a heterogeneous population that is comprised of myeloid-cell progenitors and precursors of myeloid cells. In healthy individuals, immature myeloid cells (IMCs) generated in bone marrow quickly differentiate into mature granulocytes, macrophages or dendritic cells (DCs). In pathological conditions such as cancer, various infectious diseases, sepsis, trauma, bone marrow transplantation or some autoimmune disorders, a partial block in the differentiation of IMCs into mature myeloid cells results in an expansion of this population. Importantly, the activation of these cells in a pathological context results in the upregulated expression of immune suppressive factors such as arginase (encoded by *ARG1*) and inducible nitric oxide synthase (iNOS; also known as NOS2) and an increase in the production of NO (nitric oxide) and reactive oxygen species (ROS). Together, this results in the expansion of an IMC population that has immune suppressive activity; these cells are now collectively known as MDSCs. In this

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Review, we discuss the origin, mechanism of expansion and suppressive function of MDSCs, as well as the potential to target these cells for therapeutic benefit.

Origin and subsets of MDSCs

It is important to note that MDSCs that are expanded in pathological conditions (see later) are not a defined subset of myeloid cells but rather a heterogeneous population of activated IMCs that have been prevented from fully differentiating into mature cells. MDSCs lack the expression of cell-surface markers that are specific for monocytes, macrophages or DCs and are comprised of a mixture of myeloid cells with granulocytic and monocytic morphology[6]. Early studies showed that 1–5% of MDSCs are able to form myeloid-cell colonies[7-9] and that about one third of this population can differentiate into mature macrophages and DCs in the presence of appropriate cytokines *in vitro* and *in vivo*[7-9]. In mice, MDSCs are characterized by the co-expression of the myeloid lineage differentiation antigen Gr1 (also known as Ly6G) and CD11b (also known as α_M-integrin)[10]. Normal bone marrow contains 20–30% of cells with this phenotype, but these cells make up only a small proportion (2–4%) of spleen cells and are absent from the lymph nodes in mice (Fig. 1). In humans, MDSCs are most commonly defined as $CD14^-CD11b^+$ cells or, more narrowly, as cells that express the common myeloid marker CD33 but lack the expression of markers of mature myeloid and lymphoid cells and the MHC-class-II molecule HLA-DR[11, 12]. MDSCs have also been identified within a $CD15^+$ population in human peripheral blood[13]. In healthy individuals, immature myeloid cells with described above phenotype comprise ~0.5% of peripheral blood mononuclear cells.

Recently, the morphological heterogeneity of these cells has been defined more precisely in part based on their expression of Gr1. Notably, Gr1-specific antibodies bind to both Ly6G and Ly6C, which are encoded by separate genes. However, these epitopes are recognized by different antibodies specific for each individual epitopes: anti-Ly6C and anti-Ly6G. Granulocytic MDSCs have a $CD11b^+Ly6G^+Ly6C^{low}$ phenotype, whereas MDSCs with monocytic morphology are $CD11b^+Ly6G^-Ly6C^{high}$ [6,14]. Importantly, evidence indicates that these two subpopulations may have different functions in cancer and infectious and autoimmune diseases[15-17]. During the analysis of ten different experimental tumour models, we found that both of these subsets of MDSCs were expanded. In most cases, however, the expansion of the granulocytic MDSC population was much greater than that of the monocytic subset[6] and, interestingly, the two subpopulations used different mechanisms to suppress T-cell function (see later). In addition, the ability to differentiate into mature DCs and macrophages *in vitro* has been shown to be restricted to monocytic MDSCs[6].

In recent years, several other surface molecules have been used to identify additional subsets of suppressive MDSCs, including CD80 (also known as B7.1)[18], CD115 (the macrophage colony-stimulating factor receptor)[19, 20] and CD124 (the IL-4 receptor α-chain)[20]. In our own studies, we observed that many MDSCs in tumour-bearing mice co-express CD115 and CD124[6]; however, direct comparison of MDSCs from tumour-bearing mice and $Gr1^+CD11b^+$ cells from naive mice showed that they expressed similar levels of CD115 and CD124. In addition, sorted $CD115^+$ or $CD124^+$ MDSCs from EL-4 tumour-bearing mice had the same ability to suppress T-cell proliferation on a per cell basis as did $CD115^-$ or $CD124^-$ MDSCs. This suggests that, although these molecules are associated with MDSCs, they might not be involved in the immunosuppressive function of these cells in all tumour models.

Overall, current data suggest that MDSCs are not a defined subset of cells but rather a group of phenotypically heterogeneous myeloid cells that have common biological activity.

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MDSCs in pathological conditions

MDSCs were first characterized in tumour-bearing mice or in patients with cancer. Inoculation of mice with transplantable tumour cells, or the spontaneous development of tumours in transgenic mice with tissue-restricted oncogene expression, results in a marked systemic expansion of these cells (Fig. 1 and Table 1). In addition, up to a tenfold increase in MDSC numbers was detected in the blood of patients with different types of cancer[11, 12, 21, 22]. In many mouse tumour models, as many as 20–40% of nucleated splenocytes are represented by MDSCs (in contrast to the 2-4% seen in normal mice). In addition, these cells are found in tumour tissues and in the lymph nodes of tumour-bearing mice.

Although initial observations and most of the current information regarding the role of MDSCs in immune responses has come from studies in the cancer field, accumulating evidence has shown that MDSCs also regulate immune responses in bacterial and parasitic infections, acute and chronic inflammation, traumatic stress, surgical sepsis and transplantation. A systemic expansion of both the granulocytic and monocytic subset of MDSCs was observed in mice primed with *Mycobacterium tuberculosis* as part of complete Freund's adjuvant (CFA). Acute *Trypanosoma cruzi* infection, which induces T-cell activation and increased production of interferon-γ (IFNγ), also leads to the expansion of MDSCs[23, 24]. A similar expansion of MDSCs has been reported during acute toxoplasmosis[25], polymicrobial sepsis[26], acute infection with *Listeria monocytogenes* or chronic infection with *Leishmania major*[27] and infection with helminths[28,29, 30], *Candida albicans*[31] or *Porphyromonas gingivalis*[32].

MDSC expansion is also associated with autoimmunity and inflammation. In experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, an increase in $CD11b^+Ly6C^{hi}Ly6G^-$ MDSCs was observed in the spleen and blood and these cells were found to enter the central nervous system during the inflammatory phase of the disease[16]. A significant increase in the number of MDSCs was also detected in experimental autoimmune uveoretinitis, an animal model of human intraocular inflammatory disease[33], in the skin and spleens of mice that were repeatedly treated with a contact sensitizer to induce an inflammatory response[34] and in inflammatory bowel diseases[35]. MDSCs were also found to infiltrate the spleen and suppress T-cell function in a model of traumatic stress[36]. Finally, a significant transient increase in MDSC numbers was also demonstrated in normal mice following immunization with different antigens such as ovalbumin or peptide together with CFA, a recombinant vaccinia virus expressing interleukin-2 (IL-2) or staphylococcal enterotoxin A [8, 37, 38]. Therefore, current information clearly indicates that the expansion of an immunosuppressive MDSC population is frequently observed in many pathological conditions.

Expansion and activation of MDSCs

Studies have demonstrated that the MDSC population is influenced by several different factors (Table 1), which can be divided into two main groups. The first group includes factors that are produced mainly by tumour cells and promote the expansion of MDSC through stimulation of myelopoiesis and inhibiting of the differentiation of mature myeloid cells. The second group of factors is produced mainly by activated T cells and tumour stroma, and is involved in directly activating MDSCs.

Mechanisms of MDSC expansion—Factors that induce MDSC expansion can include cyclooxygenase-2 (COX2), prostaglandins [39-41], stem-cell factor (SCF)[39], macrophage colony-stimulating factor (M-CSF), IL-6[42], granulocyte/macrophage colony-stimulating factor (GM-CSF)[41] and vascular endothelial growth factor (VEGF) [43] (Table 1). The signalling pathways in MDSCs that are triggered by most of these factors converge on Janus kinase (JAK) protein family members and signal transducer and activator of transcription 3 (STAT3) (Fig.

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2), which are signalling molecules that are involved in cell survival, proliferation, differentiation and apoptosis[44]. STAT3 is arguably the main transcription factor that regulates the expansion of MDSCs. MDSCs from tumour-bearing mice have markedly increased levels of phosphorylated STAT3 compared with IMCs from naive mice[45]. Exposure of haematopoietic progenitor cells to tumour-cell-conditioned medium resulted in the activation of JAK2 and STAT3 and was associated with an expansion of MDSCs *in vitro*, whereas inhibition of STAT3 expression in haematopoietic progenitor cells abrogated the effect of tumour-derived factors on MDSC expansion[46]. Ablation of STAT3 expression in conditional knockout mice or selective STAT3 inhibitors markedly reduced the expansion of MDSCs and increased T-cell responses in tumour-bearing mice[45, 47]. STAT3 activation is associated with increased survival and proliferation of myeloid progenitor cells, probably through upregulated expression of STAT3 target genes including B-cell lymphoma XL, (BCL-XL), cyclin D1, MYC and survivin. So, abnormal and persistent activation of STAT3 in myeloid progenitors prevents their differentiation into mature myeloid cells and thereby promotes MDSC expansion.

Recent findings suggest that STAT3 also regulates MDSC expansion through inducing the expression of S100A8 and S100A9 proteins. In addition, it has been shown that MDSCs also express receptors for these proteins on their cell surface. S100A8 and S100A9 belong to the family of S100 calcium-binding proteins that have been reported to have an important role in inflammation[48]. STAT3-dependent upregulation of S100A8 and S100A9 expression by myeloid progenitor cells prevented their differentiation and resulted in the expansion of MDSCs in the spleens of tumor-bearing and naive S100A9-transgenic mice. By contrast, MDSCs did not expand in the peripheral blood and spleens of mice deficient for S100A9 following challenge with tumour cells or CFA[49]. In a different study, S100A8 and S100A9 proteins were shown to promote MDSC migration to the tumour site through binding to carboxylated N-glycan receptors expressed on the surface of these cells [50]. Blocking the binding of S100A8 and S100A9 to their receptors on MDSCs *in vivo* with a carboxylated glycan-specific antibody reduced MDSC levels in the blood and secondary lymphoid organs of tumour-bearing mice[50]. In human colon tumour tissue, and in a mouse model of colon cancer, myeloid progenitor cells expressing S100A8 and S100A9 have been shown to infiltrate regions of dysplasia and adenoma. Furthermore, administration of a carboxylated glycan-specific monoclonal antibody (mAbGB3.1) was found to markedly reduced chronic inflammation and tumorigenesis[51]. Although the mechanisms involved require further study, these studies suggest that S100A9 and/or S100A8 proteins have a crucial role in regulating MDSC expansion, and may provide a link between inflammation and immune suppression in cancer.

Mechanisms of MDSC activation—Recently, it has become clear that the suppressive activity of MDSCs requires not only factors that promote their expansion but those that induce their activation. The expression of these factors, which are produced mainly by activated T cells and tumour stromal cells, is induced by different bacterial or viral products or as a result of tumour cell death [26]. These factors, which include IFNγ, ligands for Toll-like receptors (TLRs), IL-13, IL-4 and transforming growth factor-β (TGFβ), activate several different signalling pathways in MDSCs that involve STAT6, STAT1, and nuclear factor-κB (NF-κB) (Fig. 2).

Blockade of IFNγ, which is produced by activated T cells, abolishes MDSC-mediated T-cell suppression[17, 52]. STAT1 is the major transcription factor activated by IFNγ-mediated signalling and, in the tumour microenvironment, the upregulation of ARG1 and iNOS expression in MDSCs involved a STAT1-dependent mechanism. Indeed, MDSCs from *Stat1*$^{-/-}$ mice failed to up regulate ARG1 and iNOS expression and therefore did not inhibit T-cell responses[53]. Consistent with other findings, IFNγ produced by activated T cells and by

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MDSCs triggered iNOS expression and synergized with IL-4Rα and ARG1 pathways that have been implicated in the suppressive function of MDSCs[20].

An important role for the signalling pathway that involves IL-4 receptor α-chain (IL-4Rα) and STAT6 (which is activated by the binding of either IL-4 or IL-13 to IL-4Rα) in MDSC activation has been demonstrated in several studies. It has been shown that ARG1 expression is induced by culturing freshly isolated MDSCs or cloned MDSC lines with IL-4[54]. In addition, IL-4 and IL-13 upregulate arginase activity, which increases the suppressive function of MDSCs[55]. In line with these observations, other experiments have shown that STAT6 deficiency prevents signalling downstream of the IL-4Rα and thereby blocks the production of ARG1 by MDSCs[56]. In addition, the IL-4Rα–STAT6 pathway was also found to be involved in IL-13-induced TGFβ1 production by MDSCs in mice with sarcoma, which resulted in decreased tumour immunosurveillance[57]. This could be regulated by neutralizing both TGF-β and IL-13[57]. However, in breast tumor model IL-4Rα knockout mice retain high levels of MDSC after surgery[56]. In a different study that evaluated the separate role of TGFβ (not involving study of IL-4Rα) TGFβ-specific blocking antibody failed to reverse T-cell anergy in B-cell lymphoma *in vitro*[58]. It is possible that, the IL4Rα–STAT6 pathway might not be involved in promoting tumour immunosuppression in all tumour models.

TLRs have a central role in the activation of innate immune responses. Polymicrobial sepsis induced by the ligation and puncture of the caecum, which releases microbial products into the peritoneum and systemic circulation, was shown to result in an expansion of the MDSC population in the spleen that was dependent on the TLR adaptor molecule myeloid differentiation primary-response gene 88 (MyD88)[26]. However, wild-type mice and mice lacking a functional TLR4 protein had comparable expansion of the MDSC during polymicrobial sepsis, which suggests that signalling through TLR4 is not required for MDSC expansion and that MyD88-dependent signalling pathways that are triggered by other TLRs probably contribute to the expansion of MDSCs in sepsis[26]. This indicates that the activation of MDSCs is a fundamental outcome of the host innate immune response to pathogens that express TLR ligands.

It is important to note that an increase in the production and/or recruitment of IMCs in the context of acute infectious diseases or following vaccination does not necessarily represent an expansion of an immunosuppressive MDSC population. It is likely that under pathological conditions, the expansion of a suppressive MDSC population is regulated by two different groups of factors that have partially overlapping activity: those that induce MDSC expansion and those that induce their activation (which leads to increased levels of ROS, arginase, and/or NO). This two-tiered system may allow for flexibility in the regulation of these cells under physiological and pathological conditions.

Mechanisms of MDSC suppressive activity

Most studies have shown that the immunosuppressive functions of MDSCs require direct cell–cell contact, which suggests that they act either through cell-surface receptors and/or through the release of short-lived soluble mediators. The following sections describe the several mechanisms that have been implicated in MDSC-mediated suppression of T-cell function.

Arginase and iNOS—Historically, the suppressive activity of MDSCs has been associated with the metabolism of L-arginine. L-arginine serves as a substrate for two enzymes: iNOS, which generates NO, and arginase, which converts L-arginine into urea and L-ornithine. MDSCs express high levels of both arginase and iNOS, and a direct role for both of these enzymes in the inhibition of T-cell function is well established; this has been reviewed recently[59, 60]. Recent data suggest that there is a close correlation between the availability of arginine and the regulation of T-cell proliferation[11, 61]. The increased activity of arginase in

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MDSCs leads to enhanced L-arginine catabolism, which depletes this non-essential amino acid from the microenvironment. The shortage of L-arginine inhibits T-cell proliferation through several different mechanisms, including decreasing their CD3ζ expression[62] and preventing their upregulation of the expression of the cell cycle regulators cyclin D3 and cyclin-dependent kinase 4 (CDK4)[63]. NO suppresses T-cell function through a variety of different mechanisms that involve the inhibition of JAK3 and STAT5 in T cells[64], the inhibition of MHC class II expression [65] and the induction of T-cell apoptosis[66].

ROS—Another important factor that contributes to the suppressive activity of MDSCs is ROS. Increased production of ROS has emerged as one of the main characteristics of MDSCs in both tumour-bearing mice and patients with cancer[6, 10, 13, 53, 67-70]. Inhibition of ROS production by MDSCs isolated from mice and patients with cancer completely abrogated the suppressive effect of these cells *in vitro*[10, 13, 67]. Interestingly, ligation of integrins expressed on the surface of MDSCs was shown to contribute to increased ROS production following the interaction of MDSCs with T cells[10]. In addition, several known tumour-derived factors, such as TGFβ, IL-10, IL-6, IL-3, platelet-derived growth factor (PDGF) and GM-CSF, can induce the production of ROS by MDSCs (for review see [Ref 71]).

The involvement of ROS and NO in mechanisms of MDSC suppression are not restricted to neoplastic conditions, as inflammation and microbial products are also known to induce the development of a MDSC population that produces ROS and NO following interactions with activated T cells[15]. Similar findings were observed in models of EAE[16] and acute *Toxoplasmosis* infection [16]. In addition, it has been observed that MDSCs mediated their suppressive function through IFNγ-dependent NO production in an experimental model of *Trypanosoma cruzi* infection[23].

Peroxynitrite—More recently, it has emerged that peroxynitrite ($ONOO^-$) is a crucial mediator of MDSC-mediated suppression of T-cell function. Peroxynitrite is a product of a chemical reaction between NO and superoxide anoion (O_2^-) and is one of the most powerful oxidants produced in the body. It induces the nitration and nitrosylation of the amino acids cystine, methionine, tryptophan and tyrosine[72]. Increased levels of peroxynitrite are present at sites of MDSC and inflammatory-cell accumulation, including sites of ongoing immune reactions. In addition, high levels of peroxynitrite are associated with tumour progression in many types of cancer[72, 73,74-78], which has been linked with T-cell unresponsiveness. Bronte and colleagues reported that human prostate adenocarcinomas were infiltrated by terminally-differentiated $CD8^+$ T cells that were in an unresponsive state. High levels of nitrotyrosine were present in the T cells, which suggested the production of peroxynitrites in the tumour environment. Inhibiting the activity of arginase and iNOS, which are expressed in malignant but not in normal prostate tissue and are key enzymes of L-arginine metabolism,, led to decreased tyrosine nitration and restoration of T-cell responsiveness to tumour antigens[79]. In addition, we have demonstrated that peroxynitrite production by MDSCs during direct contact with T cells results in nitration of the T-cell receptor (TCR) and CD8 molecules, which alters the specific peptide binding of the T cells and renders them unresponsive to antigen-specific stimulation. However, the T cells maintained their responsiveness to nonspecific stimuli[80]. This phenomenon of MDSC induced antigen-specific T-cell unresponsiveness was also observed *in vivo* in tumour-bearing mice[53].

Subset-specific suppressive mechanisms?—Recent findings indicate that different subsets of MDSC might use different mechanisms by which to suppress T-cell proliferation. As described earlier, two main subsets of MDSCs have been identified: a granulocytic subset and a monocytic subset. The granulocytic subset of MDSC was found to express high levels of ROS and low levels of NO, whereas the monocytic subset expressed low levels of ROS and high levels of NO and both subsets expressed ARG1[6] (Fig.3). Interestingly, both populations

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suppressed antigen-specific T-cell proliferation to an equal extent, despite their different mechanisms of action. Consistent with these observations, Movahedi *et al.* also reported two distinct MDSC subsets in tumour-bearing mice, one that consisted of mononuclear cells that resembled inflammatory monocytes and a second that consisted of polymorphonuclear cells that were similar to immature granulocytes. Again, both populations were found to suppress antigen-specific T-cell responses, although by using distinct effector molecules and signalling pathways. The suppressive activity of the granulocytic subset was ARG1-dependent, in contrast to the STAT1- and iNOS-dependent mechanism of the monocyte fraction[17]. Finally, the same trend was observed in *Trypanosoma cruzii* infection. In this case, monocytic MDSCs produced NO and strongly inhibited T-cell proliferation, and granulocytic MDSCs produced low levels of NO and did not inhibit T-cell proliferation, although they did produce superoxide[15]. The biological significance of such functional dichotomy of these two MDSC subsets remains to be elucidated.

Induction of T_{Reg} cells—Recently, the ability of MDSCs to promote the *de novo* development of FOXP3$^+$ regulatory T (T_{Reg}) cells *in vivo* has been described[18, 19]. The induction of T_{Reg} cells by MDSCs was found to require the activation of tumour-specific T-cells and the presence of IFNγ and IL-10 but was independent of NO[19]. In mice bearing 1D8 ovarian tumours, the induction of T_{Reg} cells by MDSCs required the expression of cytotoxic lymphocyte antigen 4 (CTLA-4; also known as CD152) by MDSCs[18]. In a mouse model of lymphoma, MDSCs were shown to induce T_{Reg}-cell expansion through a mechanism that required arginase and the capture, processing and presentation of tumour-associated antigens by MDSCs, but not TGFβ[58]. By contrast, Movahedi *et al.* found that the percentage of T_{Reg} cells was invariably high throughout tumour growth and did not relate to the kinetics of expansion of the MDSC population, suggesting that MDSCs were not involved in T_{Reg}-cell expansion[17]. Furthermore, in a rat model of kidney allograft tolerance that was induced with a CD28-specific antibody, MDSCs that were co-expressing CD80 and CD86 were found to have a limited effect on the expansion of the T_{Reg}-cell population[81]. Although further work is required to resolve these discrepancies and to determine the physiological relevance of these studies, it seems possible that MDSCs are involved in T_{Reg}-cell differentiation through the production of cytokines or direct cell–cell interactions. Furthermore, MDSCs and T_{Reg} cells might be linked in a common immunoregulatory network (see later).

Tissue-specific effects on MDSCs

A major unresolved question in this field is whether MDSCs mediate antigen-specific or nonspecific suppression of T-cell responses. Provided that MDSCs and T cells are in close proximity, the factors that mediate MDSC suppressive function (ROS, arginase and NO) can inhibit T-cell proliferation regardless of the antigen specificity of the T cells. Indeed, numerous *in vitro* studies have demonstrated the antigen nonspecific nature of MDSC-mediated suppression of T cells[82 83]. However, whether the situation is the same *in vivo* is not clear, and evidence suggests that MDSC-mediated immunosuppression in peripheral lymphoid organs is mainly antigen-specific. The idea that MDSC-mediated T-cell suppression occurs in an antigen-specific manner is based on findings that antigen-specific interactions between antigen-presenting cells and T cells result in much more stable and more prolonged cell–cell contact than nonspecific interactions[82, 84, 85]. Such stable contacts are necessary for MDSC-derived ROS and peroxynitrite to mediate effects on the molecules on the surface of T cells that render the T cells unresponsive to specific antigen. It should be noted that such modification of cell-surface molecules does not lead to T-cell death nor prevent nonspecific T-cell activation. Other evidence that supports the idea that MDSCs mediate antigen-specific suppression is the finding that that MDSCs can take up soluble antigens, including tumour-associated antigens, and process and present them to T cells[17 80]; blockade of MDSC–T-cell interactions with a MHC-class-I-specific antibody abrogated MDSC-mediated inhibition of T-

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cell responses *in vitro*[86]. The MHC-class-I-restricted nature of MDSC-mediated $CD8^+$ T-cell suppression has also been demonstrated *in vivo* in tumor models[53] and in the model of inflammatory bowel disease [35]. This is consistent with the recent observation that large numbers of tumour-induced MDSCs did not inhibit $CD8^+$ T-cell responses specific for unrelated antigens in a model of sporadic cancer[87]. Notably, it is currently unclear whether similar antigen-specific mechanisms of MDSC-mediated suppression operate on $CD4^+$ T cells, as published studies have only assessed the effects of MDSCs on $CD8^+$ T cells. Addressing this question is complicated by the fact that only a small proportion of MDSCs in many tumour models expresses MHC class II molecules.

The theory that MDSCs suppress T-cell responses in an antigen-specific manner helps to explain the finding that T cells in the peripheral lymphoid organs of tumour-bearing mice and in the peripheral blood of cancer patients can still respond to stimuli other than tumour-associated antigens, including viruses, lectins, co-stimulatory molecules, IL-2 and CD3- and CD28-specific antibodies[21, 80, 88-90]. Furthermore, even patients with advanced stage cancer do not have systemic immunodeficiency except in cases in which the patient has received high doses of chemotherapy or is at a terminal stage of the disease.

Evidence suggets that the nature of MDSC-mediated suppression at the tumour site is quite different to that which occurs in the periphery. MDSCs actively migrate into the tumour site[10], where they upregulate the expression of ARG1 and iNOS, downregulate the production of ROS and/or rapidly differentiate into tumour-associated macrophages (TAMs) [52]. The levels of NO and arginase produced by tumour-associated MDSCs and TAMs are much higher than those of MDSCs found in peripheral lymphoid organs of the same animals. In addition, TAMs produce several cytokines (reviewed in REFs[91, 92]) that suppress T-cell responses in a nonspecific manner (Fig. 4). The mechanisms by which MDSC functions are regulated within the tumour microenvironment, and how they differ from those that operate at peripheral sites, remain unclear. It is possible that tumour stroma, hypoxia and/or the acidophilic environment have a role.

Therapeutic targeting of MDSCs

The recognition that immune suppression has a crucial role in promoting tumour progression and contributes to the frequent failure of cancer vaccines to elicit an immune response has resulted in a paradigm shift with respect to approaches for cancer immunotherapy. Indeed, it has become increasingly clear that successful cancer immunotherapy will be possible only with a strategy that involves the elimination of suppressive factors from the body. As MDSCs are one of the main immunosuppressive factors in cancer and other pathological conditions, several different therapeutic strategies that target these cells are currently being explored (Table 2). Although the studies described below were carried out in tumor-bearing hosts, it is likely that the same strategies will be useful in other pathological conditions in which inhibition or elimination of MDSCs is a therapeutic aim.

Promoting myeloid-cell differentiation—One of the most promising approaches by which to target MDSCs for therapy is to promote their differentiation into mature myeloid cells that do not have suppressive abilities. Vitamin A has been identified as a compound that can mediate this effect: vitamin A metabolites such as retinoic acid have been found to stimulate the differentiation of myeloid progenitors into DCs and macrophages [86, 93]. Mice that are deficient in vitamin A[94] or that have been treated with a pan-retinoic-acid-receptor antagonist[95], show an expansion of MDSCs in the bone marrow and spleen. Conversely, therapeutic concentrations of all-trans retinoic acid (ATRA) results in substantial decrease in the presence of MDSCs in cancer patients and tumour-bearing mice. ATRA induced MDSCs to differentiate into DCs and macrophages *in vitro* and *in vivo* [12, 86, 96]. It is probable that

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ATRA preferentially induces the differentiation of the monocytic subset of MDSCs, whereas it causes apoptosis of the granulocytic subset. The main mechanism of ATRA-mediated differentiation involved an upregulation of glutathione synthesis and a reduction in ROS levels in MDSCs [97]. Decreasing the number of MDSCs in tumour-bearing mice resulted in increased tumour-specific T-cell responses, and the combination of ATRA and two different types of cancer vaccine prolonged the anti-tumour effect of the vaccine treatment in two different tumour models [96]. Moreover, administration of ATRA to patients with metastatic renal cell carcinoma resulted in a substantial decrease in the number of MDSCs in the peripheral blood and improved antigen-specific response of T cells [21]. Further studies will lead to identification of other agents that have a similar effect. So far, evidence suggests that Vitamin D3 may be another agent with the potential to decrease MDSC numbers in patients with cancer, as it is also known to promote myeloid-cell differentiation[98].

Inhibition of MDSC expansion—Because MDSC expansion is known to be regulated by tumour-derived factors (Table 1), several studies have focused on neutralizing the effects of these factors. Recently, SCF has been implicated in causing MDSC expansion in tumour-bearing mice[39]. Inhibition of SCF-mediated signalling by blocking its interaction with its receptor, c-kit, decreased MDSC expansion and tumor angiogenesis[39]. VEGF, another tumour-derived factor that is involved in promoting MDSC expansion, might also be a useful target by which to manipulate MDSC. However, in a clinical trial of 15 patients with refractory solid tumours, treatment with VEGF–trap (a fusion protein that binds all forms of VEGF-A and placental growth factor) showed no effect on MDSC numbers and did not result in increased T-cell responses[99]. By contrast, treatment of patients with metastatic renal cell cancer with a VEGF-specific blocking antibody (known as avastin) resulted in a decrease in the size of a $CD11b^{+}VEGFR1^{+}$ population of MDSCs in the peripheral blood [100]. However, whether avastatin treatment resulted in an improvement in antitumour responses in these patients has not been determined. Finally, inhibition of matrix metalloproteinase 9 function in tumor-bearing mice decreased the number of MDSCs in the spleen and tumour tissues and resulted in a significant delay in the growth of spontaneous NeuT tumours in transgenic BALB/c mice[101]. However, the mechanism responsible for this outcome remains to be elucidated.

Inhibition of MDSC function—Another approach by which to inhibit MDSCs is to block the signalling pathways that regulate the production of suppressive factors by these cells. One potential target by which this might be achieved is COX2. COX2 is required for the production of prostaglandin E2, which in 3LL tumour cells[61] and mammary carcinoma[40] has been shown to induce the upregulation of ARG1 expression by MDSCs, thereby inducing their suppressive function. Accordingly, COX2 inhibitors were found to downregulate the expression of ARG1 by MDSCs, which improved antitumour T-cell responses and enhanced the therapeutic efficacy of immunotherapy[102, 103]. Similarly, phosphodiesterase-5 inhibitors such as sildenafil were found to downregulate the expression of arginase and iNOS expression by MDSCs, thereby inhibiting their suppressive function in growing tumours[104]. This resulted in the induction of a measurable anti-tumour immune response and a marked delay of tumour progression in several mouse models [104].

ROS inhibitors have also been shown to be effective for decreasing MDSC-mediated immune suppression in tumour-bearing mice. The coupling of a NO-releasing moiety to a conventional non-steroidal anti-inflammatory drug has proven to be an efficient means by which to inhibit the production of ROS. One such drug, nitroaspirin, was found to limit the activity of ARG1 and iNOS in spleen MDSCs[105]. In combination with vaccination with endogenous retroviral gp70 antigen, nitroaspirin inhibited MDSCs function and increased the number and function of tumour-antigen-specific T cells[105].

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Elimination of MDSCs—MDSCs can be directly eliminated in pathological settings by using some chemotherapeutic drugs. Administration of one such drug, gemcitabine, to mice that were bearing large tumours resulted in a dramatic reduction in the number of MDSCs in the spleen and resulted in a marked improvement in the anti-tumour response induced by immunotherapy[106, 107]. This effect was specific to MDSCs, as a significant decrease in the number of T or B cells was not observed in these animals. Furthermore, in a study of 17 patients with early-stage breast cancer that were treated with doxorubicin–cyclophosphamide chemotherapy, a decrease in the level of MDSCs in the peripheral blood was observed[22].

Evidence suggests that there is a broad range of methods that will be effective for targeting of the number and/or function of MDSCs *in vivo*. These strategies will undoubtedly help to further investigate the biology of these cells as well as expedite clinical applications to treat cancer and other pathological conditions.

MDSCs as regulatory myeloid cells?

The wealth of information that has accumulated in recent years regarding the biology of MDSCs suggests that these cells might have evolved as a regulatory component of the immune system. These cells are absent under physiological conditions, as IMCs in naive mice are an intrinsic part of normal haematopoiesis that are not immunosuppressive in an unactivated state. In conditions of acute stress, infection or immunization, there is a transient expansion of this IMC population, which then quickly differentiates into mature myeloid cells. This transient IMC population can mediate the suppressive functions that are characteristic of MDSCs but, because the acute conditions are short-lived, the suppressive functions of this transient population have a minimal impact on the overall immune response. However, these cells probably function as important ‘gatekeepers’ that prevent pathological immune-mediated damage.

The role of the MDSC population in settings of chronic infections and cancer is very different. In these pathological conditions, the prolonged and marked expansion of IMCs and their subsequent activation leads to the expansion of a large population of MDSCs with immunosuppressive abilities. MDSCs accumulate in peripheral lymphoid organs and migrate to tumour sites, where they contribute to immunosuppression. Furthermore, some evidence suggests that MDSCs can also induce expansion of regulatory T cells. Future studies will reveal whether MDSCs can be considered part of a natural immune regulatory network.

Concluding remarks

The field of MDSC research has more outstanding questions than answers. The roles of specific MDSC subsets in mediating T-cell suppression, and the molecular mechanisms responsible for inhibition of myeloid-cell differentiation, need to be elucidated. The issue of whether T-cell suppression occurs in an antigen-specific manner remains to be clarified, as do the mechanisms that cause MDSC migration to peripheral lymphoid organs. Some of the main priorities in this field should include a better characterization of human MDSCs and a clear understanding of whether targeting these cells in patients with various pathological conditions will be of clinical significance. Conversely, adoptive cellular therapy with MDSCs may be an attractive opportunity by which to inhibit immune responses in the setting of autoimmune disease or transplantation. The challenge for these approaches will be to devise methods by which to generate these cells *ex vivo* in clinical-grade conditions such that they are suitable for administration to patients. If the past 5–6 years are an indication of the potential for progress in this area, it is safe to estimate that there will soon be significantly more discoveries that further our understanding about the biology and clinical utility of MDSCs.

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Box 1. Definition of myeloid-derived suppressor cells (MDSCs)

- a heterogeneous population of cells of myeloid origin that consist of myeloid progenitors and immature macrophages, immature granulocytes and immature dendritic cells
- present in activated state that is characterized by the increased production of reactive oxygen and nitrogen species, and of arginase
- potent suppressors of various T-cell functions
- in mice, their phenotype is $CD11b^{+}Gr1^{+}$, although functionally distinct subsets within this population have been identified (see main text)
- in humans, their phenotype is $Lin^{-}HLA^{-}DR^{-}CD33^{+}$ or $CD11b^{+}CD14^{-}CD33^{+}$. Human cells do not express a marker homologous to mouse Gr1. MDSC have also been identified within a $CD15^{+}$ population in human peripheral blood.
- in the steady state, immature myeloid cells lack suppressive activity and are present in the bone marrow, but not in secondary lymphoid organs
- accumulation of MDSCs in lymphoid organs and in tumours in response to various growth factors and cytokines is associated with various pathological conditions (most notably cancer)
- in tumour tissues, MDSCs can be differentiated from tumour-associated macrophages (TAMs) by their high expression of Gr1 (not expressed by TAMs) by their low expression of F4/80 (expressed by TAMs), by the fact that a large proportion of MDSCs have a granulocytic morphology and based the upregulated expression of both arginase and inducible nitric oxide synthase by MDSCs but not TAMs.

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Glossary

Term	Definition
Sepsis	A systemic response to severe infection or tissue damage, leading to a hyperactive and unbalanced network of pro-inflammatory mediators. Vascular permeability, cardiac function and metabolic balance are affected, resulting in tissue necrosis, multi-organ failure and death
Complete Freund's adjuvant (CFA)	An oil that contains an emulsifying agent and killed mycobacteria, which increase the immune response to an immunogen. For administration, a water-in-oil emulsion is made with a solution that contains the immunogen of interest
Experimental autoimmune encephalomyelitis (EAE)	An animal model of the human autoimmune disease multiple sclerosis. EAE is induced in experimental animals by immunization with myelin or peptides derived from myelin. The animals develop a paralytic disease with inflammation and demyelination in the brain and spinal cord
Myelopoiesis	The process of differentiation of common myeloid progenitor to polymorphonuclear leucocytes and monocytes
NADPH oxidase complex	An enzyme system that consists of multiple cytosolic and membrane-bound subunits. The complex is assembled in activated neutrophils mainly on the phagolysosomal membrane. NADPH oxidase uses electrons from NADPH to reduce molecular oxygen to form superoxide anions. Superoxide anions are enzymatically converted to hydrogen peroxide, which is converted by myeloperoxidase to hypochloric acid, a highly toxic and microbicidal agent
Tumour immunosurveillance	The process of the host immune system to recognize tumor antigens and eliminate the tumors
Regulatory T cells (T_{Reg} cells)	A specialized type of $CD4^+$ T cell that can suppress the responses of other immune cells. These cells provide a crucial mechanism for the maintenance of peripheral self-tolerance and are characterized by expression of CD25 (also known as the α-chain of the interleukin-2 receptor) and the transcription factor forkhead box P3 (FOXP3).
Tumour-associated macrophages (TAMs).	These cells differentiate from circulating blood monocytes and MDSC that have infiltrated tumours. These cells can have positive or negative effects on tumorigenesis (that is tumour promotion or immunosurveillance, respectively)

Biographies

Dmitry I. Gabrilovich is a Robert Rothman Endowed Chair in Cancer Research, Head of the section of Dendritic Cell Biology at the H. Lee Moffitt Cancer Center and Professor at University of South Florida, USA. His lab studies myeloid-derived suppressor cells and dendritic cells in cancer as well as the molecular and cellular mechanisms of myeloid-cell differentiation in the bone marrow.

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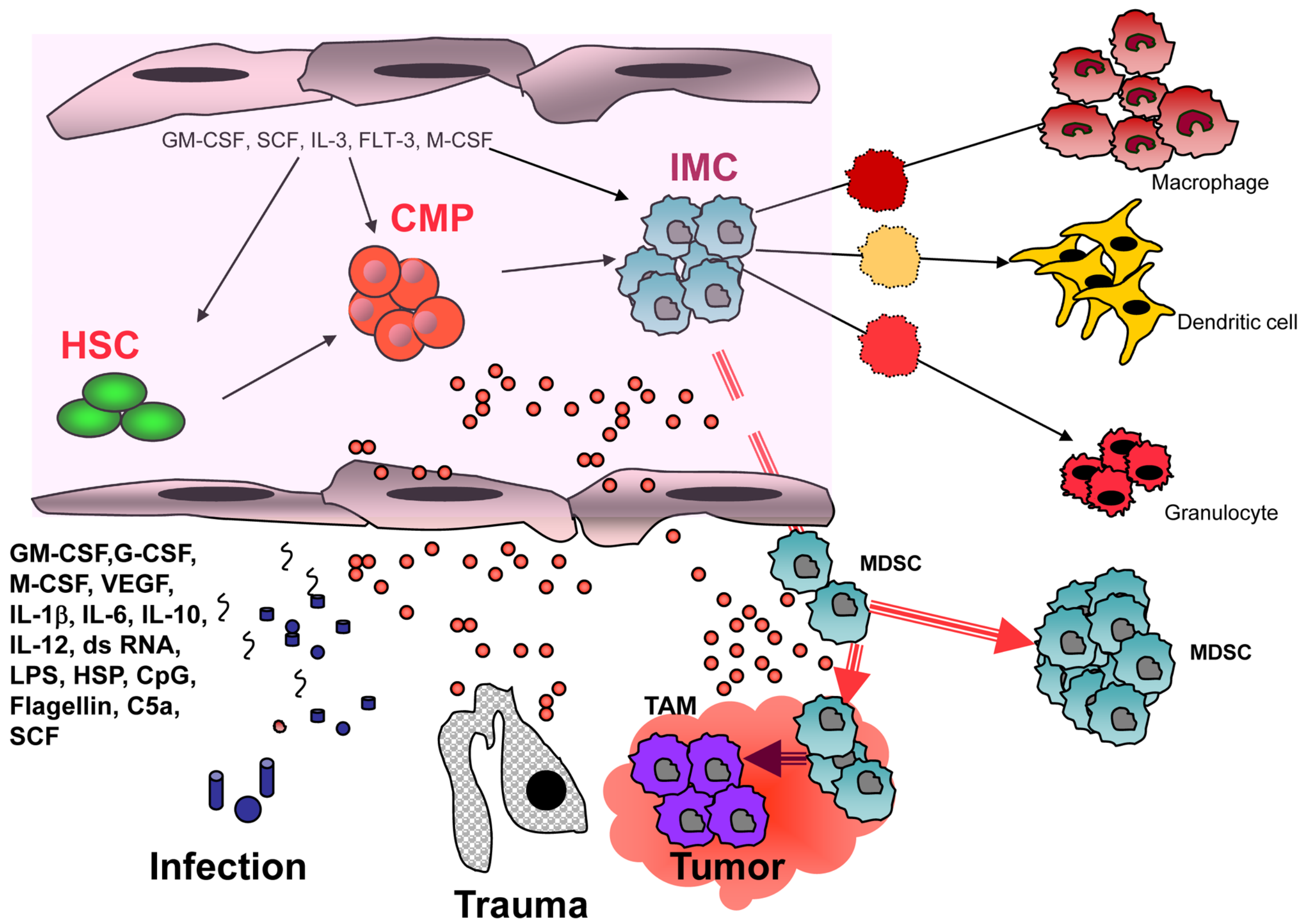

Figure 1. The origin of MDSCs

Immature myeloid cells (IMCs) are part of the normal process of myelopoiesis, which takes place in the bone marrow and is controlled by a complex network of soluble factors that include cytokines such as granulocyte/macrophage colony-stimulating factor (GM-CSF), stem-cell factor (SCF), interleukin-3 (IL-3), FMS-related tyrosine kinase 3 (FLT-3), macrophage colony-stimulating factor (M-CSF) and cell-expressed molecules including Notch (not shown). Haematopoietic stem cells (HSCs) differentiate into common myeloid progenitor (CMP) cells and then into IMCs. Normally, IMCs migrate to different peripheral organs, where they differentiate into dendritic cells, macrophages and/or granulocytes. However, factors produced in the tumour microenvironment and/or during acute or chronic infections, trauma or sepsis, promote the accumulation of IMCs at these sites, prevent their differentiation and induce their activation. These cells exhibit immunosuppressive functions and are therefore known as myeloid-derived suppressor cells (MDSCs). MDSCs can also differentiate into tumor-associated macrophages (TAMs) within the tumour environment, which are cells that have a phenotype and function that is distinct from MDSCs.

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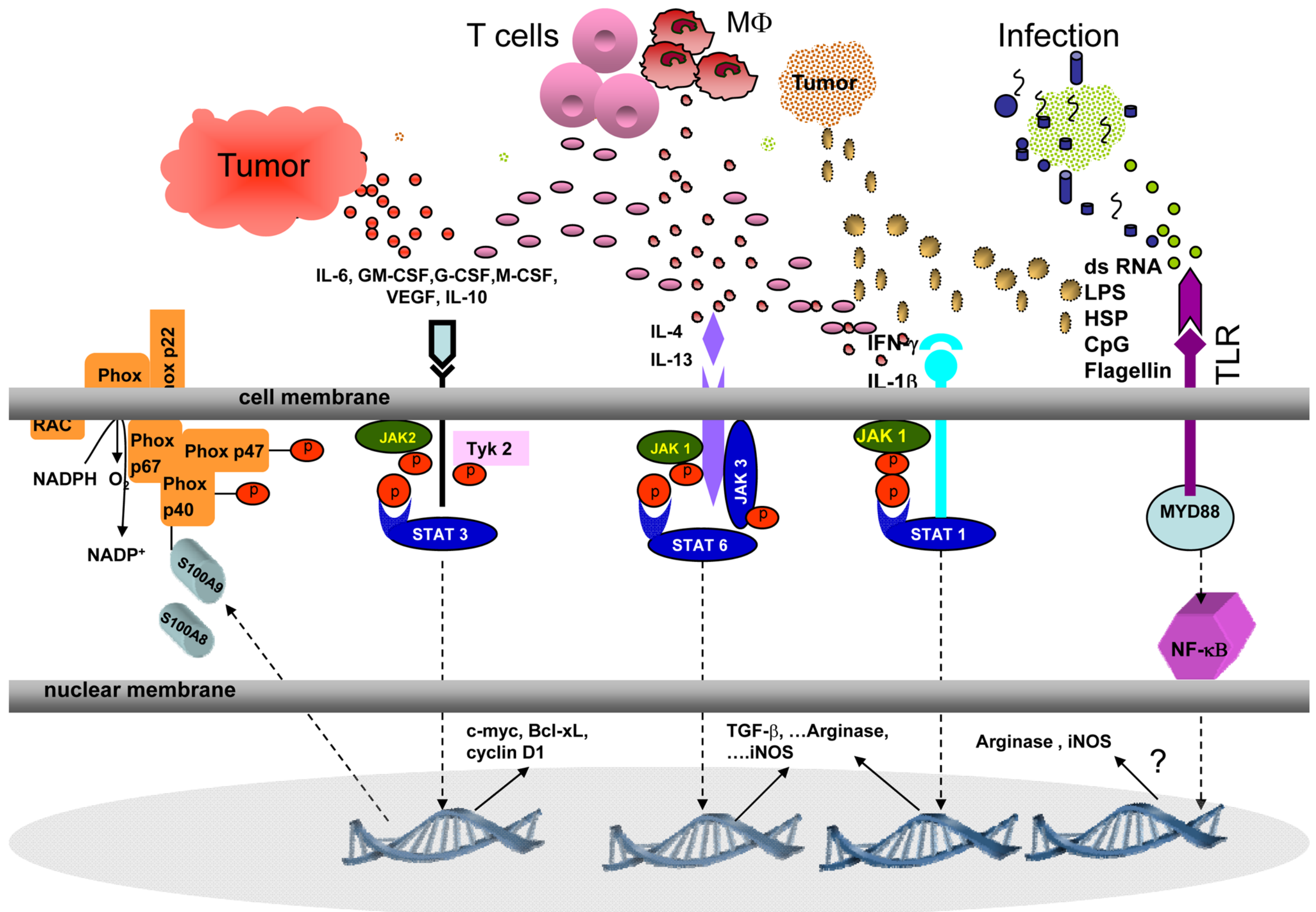

Figure 2. Signalling pathways involved in the expansion of MDSC populations

The accumulation of myeloid-derived suppressor cells (MDSCs) is regulated by several factors that are released by tumour cells, tumour stromal cells, activated T cells and macrophages, apoptotic tumour cells, bacterial and viral agents and by pathogen-infected cells. These factors trigger several different signalling pathways in MDSCs that mainly involve the STAT (signal transducer and activator of transcription) family of transcription factors. STAT3 regulates the expansion of MDSCs by stimulating myelopoiesis and inhibiting myeloid-cell differentiation. It also contributes to the increased production of reactive oxygen species (ROS) by MDSCs. The activation of STAT6 and STAT1, as well as TLR-mediated activation of nuclear factor-κB (NF-κB), by these factors results in the activation of MDSCs, which leads to the upregulation of iNOS and arginase and increased production of suppressive cytokines such as transforming growth factor-β (TGF-β). In combination with STAT3 they also contribute to upregulation of ROS production by these cells. S100A8 and S100A9 directly bind to p67phox and p47phox, which are crucial components of NADPH complex. This binding potentiates NADPH oxidase activation in MDSCs, which causes increased production of ROS, leading to the observed suppressive effects. It is likely that MDSC activation via TLR play especially important role during pathogenic infections.

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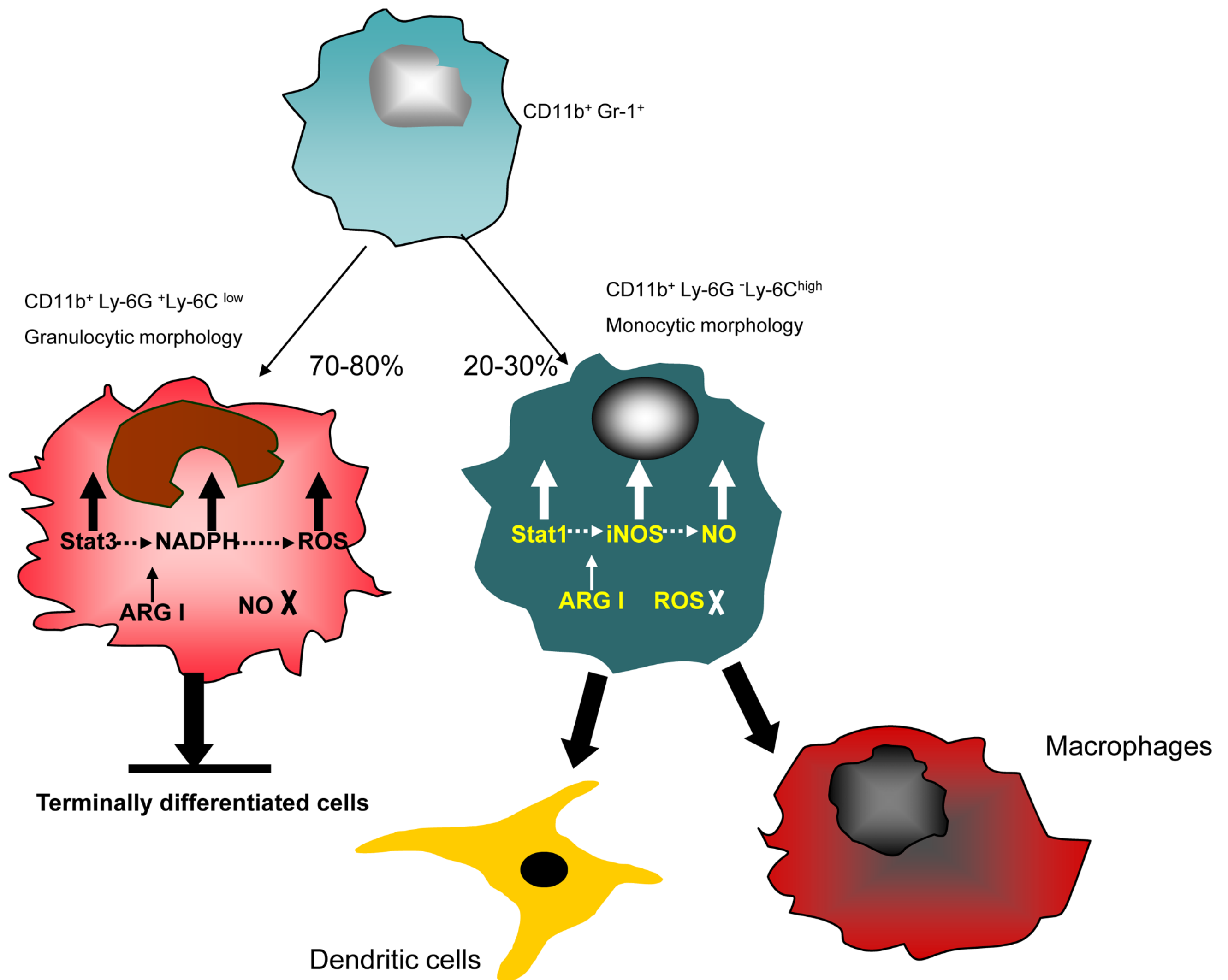

Figure 3. Suppressive mechanisms mediated by different subsets of MDSCs

Myeloid-derived suppressor cells (MDSCs) consist of two major subsets: granulocytic MDSCs with a $CD11b^{+}Ly6G^{+}Ly6C^{low}$ phenotype and monocytic MDSCs with a $CD11b^{+}Ly6G^{-}Ly6C^{high}$ phenotype. In most tumour models, it is predominantly (70–80%) the granulocytic subset of MDSCs that expands. We hypothesize that the granulocytic subset of MDSCs has increased activity of STAT3 (signal transducer and activator of transcription 3) and NADPH, which results in high levels of reactive oxygen species (ROS) but little nitric oxide (NO) production. ROS and, in particular, peroxynitrite (the product of a chemical reaction between superoxide and NO) induces post-translational modification of T-cell receptors and may cause antigen-specific T-cell unresponsiveness. The monocytic MDSC subset has upregulated expression of STAT1 and inducible nitric oxide synthase (iNOS) and increased levels NO but little ROS production. NO, which is produced by the metabolism of L-arginine by iNOS, suppresses T-cell function through a variety of different mechanisms that involve the inhibition of Janus kinase 3 (JAK3) and STAT5, the inhibition of MHC class II expression and the induction of T-cell apoptosis. Both subsets have elevated level of arginase 1 (ARG1) activity that causes T-cell suppression through depletion of arginine. Only monocytic MDSCs can differentiate into mature dendritic cells and macrophages *in vitro*.

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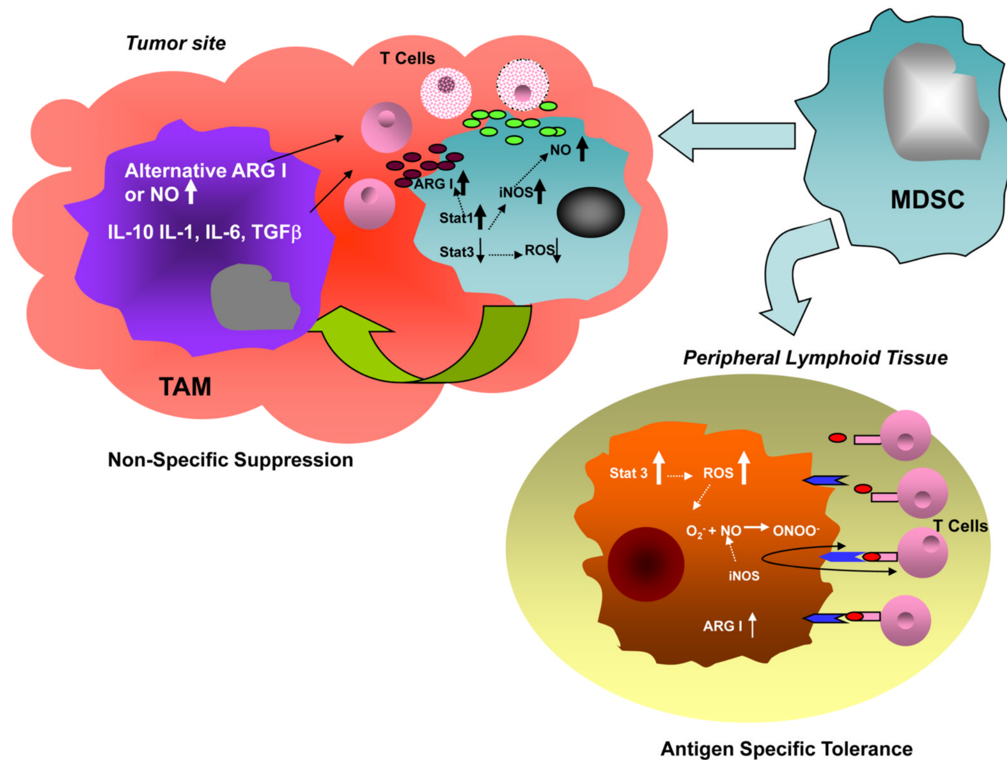

Figure 4. The mechanisms of MDSC-mediated immune suppression differ in lymphoid organs and at the site of a tumour

Myeloid-derived suppressor cells (MDSCs) migrate to tumour sites and peripheral lymphoid organs. (a) In peripheral lymphoid organs, MDSCs produce high levels of reactive oxygen species (ROS), including peroxynitrite, and upregulate signal transducer and activator of transcription 3 (STAT3) activity. This is associated with a moderate increase in arginase activity and relatively low level of nitric oxide (NO) production. MDSCs are able to take up, process and present antigens to antigen-specific $CD8^+$ T cells. During this close cell–cell contact, peroxynitrite produced by MDSCs causes nitration and nitrosylation of different amino acids in the T-cell receptor (TCR) and CD8 molecules on the surface of T cells, which causes the T cells to become unresponsive to specific antigen stimulation. However, these cells retain the ability to respond to non-specific stimulation with CD3- and CD28-specific antibodies. (b) By contrast, MDSCs that migrate to the tumour site upregulate STAT1 activity, produce high levels of inducible nitric oxide synthase (iNOS), NO and arginase. This is associated with low levels of ROS. High amounts of arginase and NO that are released from MDSCs inhibit T-cell function in a nonspecific manner. MDSCs at the tumour site can also differentiate to tumour-associated macrophages (TAMs). In contrast to MDSCs, TAMs upregulate the expression of either arginase or iNOS, depending on the nature of the tumour microenvironment (see [REF 91]), but not of both proteins. TAMs acquire ability to produce several suppressive cytokines. Together with MDSCs, TAMs contribute to nonspecific T-cell suppression in the tumour microenvironment.

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Table 1
Factors implicated in the expansion of MDSCs in cancer

Factor	Tumour model (mice)	Type of cancer (humans)
VEGF [43] [108, 109] [100] [110-112]	Breast cancer Sarcoma Melanoma Lymphoma Lung carcinoma	Breast cancer Renal cell cancer Pancreatic cancer
GM-CSF [113, 114] [41, 113, 115, 116] [117] [118]	Lewis lung carcinoma Colon carcinoma Mammary adenocarcinoma TS/A tumour	Melanoma
G-CSF[119]	Lewis lung carcinoma Metha Sarcoma Melanoma	ND
M-CSF [96, 120]	Sarcoma Mammary carcinoma	Human renal cell carcinoma cell lines
Gangliosides [121] [122]	Neuroblastoma Glioma	ND
Prostaglandins [40] [61] [102]	Mammary carcinoma Lung cancer Renal cancer Colon cancer	ND
IFNγ [20, 123-125]	Mammary adenocarcinoma Fibrosarcoma Colon carcinoma Lymphoma	ND
C5a[126]	Cervical Cancer /Lung tumor	ND
SCF [39]	Colon carcinoma	
S100A8 and S100A9[49, 50]	Colon carcinoma Lymphoma Fibrosarcoma Mammary carcinoma	ND
TGF-β [57, 127-129]	Colon carcinoma Fibrosarcoma Mammary adenocarcinoma	Head and neck cancer
IL-1-β [130, 131]	Fibrosarcoma, Mammary carcinoma	ND
IL-6 [42]	Mammary carcinoma	ND

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Factor	Tumour model (mice)	Type of cancer (humans)
IL-10. [19]. [40].	Colon Cancer Melanoma Mammary carcinoma	ND
IL-12 [9]	Colon Cancer	ND
IL-13 [20, 57]	Colon carcinoma Fibrosarcoma Mammary adenocarcinoma Lymphoma	ND
MMP-9 [101, 132]	Colon carcinoma, Lewis Lung carcinoma Mammary carcinoma	ND
CCL2 [17, 119, 125, 133]	Lewis lung carcinoma MethA sarcoma Melanoma Lymphoma	ND
CXCL5/12 [127]	Mammary adenocarcinoma	ND

C5a, complement component 5a; CCL2, CC-chemokine ligand 2; CXCL5, CXC-chemokine ligand 5; G-CSF, granulocyte-stimulating factor; GM-CSF, granulocyte/macrophage colony stimulating factor; IL-1β interleukin-1β; IFNγ, interferon-γ; M-CSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase 9; ND, not determined; SCF, stem cell factor; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

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Table 2

Therapeutic strategies to target MDSCs

Therapeutic agents	Type of cancer tested	References
Cyclooxygenase 2 inhibitor (SC58236)	Mammary carcinoma (mice)	40
Amino-biphosphonate	Mammary tumors (mice)	101
Biphosphanate, sildenafil and tadalafil	Mammary carcinoma(mice) Colon Carcinoma (mice) Fibrosarcoma (mice)	104
KIT-specific antibody	Colon Carcinoma (mice)	39
Nitroaspirin	Colon Carcinoma (mice)	105
All-trans retinoic acid	Sarcoma, colon carcinioma (mice) Metastatic renal cell carcinoma (humans)	96 21
Vitamin D_3	Head and neck cancer (humans)	98
Gemcitabine	Lung cancer (mice)	106
VEGF–trap* VEGF-specific antibody (avastin)	Solid tumors (humans) Metastatic renal cell cancer (humans)	89 100
Doxorubicin and cyclophosphamide	Breast cancer (humans)	22

* **VEGF-trap is** a fusion protein that binds all forms of VEGF-A and placental growth factor