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 expand during cancer, inflammation and infection, and that have 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.

Sepsis
A systemic response to severe infection or tissue damage that leads 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.

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Suppressive myeloid cells were described more than 20 years ago in patients with cancer[1–3], but their functional importance in the immune system has only recently been appreciated. Indeed, accumulating evidence has now shown that a population of cells with suppressive activity (known as myeloid-derived suppressor cells (MDSCs)) contributes to the negative regulation of immune responses during cancer and other diseases. Features that are common to all MDSCs are their myeloid origin, their 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 MDSCs have also been described, such as the promotion of tumour angiogenesis and metastasis. However, as these non-immunological aspects of MDSC biology have recently been reviewed elsewhere[5], they will not be discussed further in this Review.

MDSCs are a heterogeneous population of cells that consists of myeloid progenitor cells and immature myeloid cells (IMCs). In healthy individuals, IMCs that are generated in the bone marrow quickly differentiate into mature granulocytes, macrophages or dendritic cells (DCs). By contrast, in pathological conditions, such as cancer, various infectious diseases, sepsis, trauma, bone marrow transplantation and some autoimmune diseases, a partial block in the differentiation of IMCs into mature myeloid cells results in the expansion of this population. Importantly, the activation of IMCs in pathological conditions results in the upregulation of their expression of immune suppressive factors, such as arginase 1 (encoded by *ARG1*) and inducible nitric oxide synthase (iNOS; also known as NOS2), as well as an increase in their production of NO (nitric oxide) and reactive oxygen species (ROS). This results in the expansion of an IMC population that has immune suppressive activity; these cells are now collectively known as MDSCs. In this Review, we discuss the origin, the mechanisms of expansion and the suppressive function of MDSCs, as well as the potential to target these cells for therapeutic benefit.

Origin and subsets of MDSCs

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 specifically expressed by monocytes, macrophages or DCs, and comprise a mixture of myeloid cells that have the morphology of granulocytes or monocytes[6]. Early studies showed that 1–5% of MDSCs can 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 the appropriate cytokines *in vitro* and *in vivo*[7–9]. In mice, MDSCs are characterized by the co-expression of the myeloid-cell lineage differentiation antigen GR1 and CD11b (also known as αM-integrin)[10]. Normal mouse 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 (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 of 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, IMCs constitute ~0.5% of peripheral blood mononuclear cells[12].

Box 1 | **Definition of MDSCs**

- Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells of myeloid origin that comprises myeloid progenitor cells and immature macrophages, immature granulocytes and immature dendritic cells.
- They are present in an activated state that is characterized by the increased production of reactive oxygen and nitrogen species, and of arginase 1.
- They are potent suppressors of various T-cell functions.
- In mice, the phenotype of MDSCs is $CD11b^+GR1^+$, although functionally distinct subsets within this population have been identified (see main text).
- In humans, the phenotype of MDSCs is $LIN^-HLA\text{-}DR^-CD33^+$ or $CD11b^+CD14^-CD33^+$; human cells do not express a marker that is homologous to mouse GR1. MDSCs 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 several pathological conditions (most notably, cancer).
- In tumour tissues, MDSCs can be distinguished 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 on the upregulated expression of both arginase 1 and inducible nitric oxide synthase (not expressed by TAMs).

Recently, the morphological heterogeneity of MDSCs has been defined more precisely in mice based, in part, on their expression of GR1. Antibodies that are specific for GR1 bind to two epitopes, LY6G and LY6C (encoded by different genes), each of which can be detected separately through the use of antibodies that are specific for each individual epitope. The use of these epitope-specific antibodies has led to the identification of two MDSC subsets: granulocytic MDSCs have a $CD11b^+LY6G^+LY6C^{low}$ phenotype, whereas MDSCs with monocytic morphology are $CD11b^+LY6G^-LY6C^{hi}$ (REFS 6,14). Importantly, evidence indicates that these two subsets 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 MDSC subsets expanded, but in most cases the expansion of granulocytic MDSCs was much greater than that of the monocytic subset[6]. Furthermore, the two MDSC subsets 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 (also known as macrophage colony-stimulating factor receptor (M-CSF) and CSF1)[19,20] and CD124 (also known as interleukin-4 receptor α-chain (IL-4Rα))[20]. In our own studies, we observed that many MDSCs in tumour-bearing mice co-expressed CD115 and CD124 (REF. 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 EL4 tumour-bearing mice had the same ability to suppress T-cell proliferation on a per cell basis as $CD115^-$ or $CD124^-$ MDSCs. This suggests that, although these molecules are associated with MDSCs, they might not be involved in their immunosuppressive function 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.

MDSCs in pathological conditions

MDSCs were first characterized in tumour-bearing mice and in patients with cancer. These cells have been shown to markedly expand systemically when mice are inoculated with transplantable tumour cells and when tumours spontaneously develop in transgenic mice with tissue-restricted oncogene expression (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 several mouse tumour models, as many as 20–40% of nucleated splenocytes are MDSCs (in contrast to the 2–4% seen in normal mice). In addition, MDSCs are found in tumour tissues and in the lymph nodes of tumour-bearing mice.

Although initial observations and most of the current information on the role of MDSCs in immune responses has come from studies in the field of cancer research, accumulating evidence has shown that MDSCs also regulate immune responses during bacterial and parasitic infections, acute and chronic inflammation, traumatic stress, sepsis and transplantation.

Indeed, a systemic expansion of both the granulocytic and monocytic subsets of MDSCs was observed in mice primed with *Mycobacterium tuberculosis* in the form of complete Freund's adjuvant (CFA). Furthermore, acute *Trypanosoma cruzi* infection, which induces T-cell activation and increased the 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*, 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, a mouse model of multiple sclerosis, an increase in the number of MDSCs (specifically the $CD11b^+LY6G^-LY6C^{hi}$ monocytic subset) 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 great 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 to suppress T-cell function in a model of traumatic stress[36]. Finally, a significant transient increase in MDSC numbers was also observed in normal mice following immunization with different antigens, including ovalbumin or peptide

Complete Freund's adjuvant
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 that are derived from myelin. The animals develop a paralytic disease with inflammation and demyelination in the brain and spinal cord.

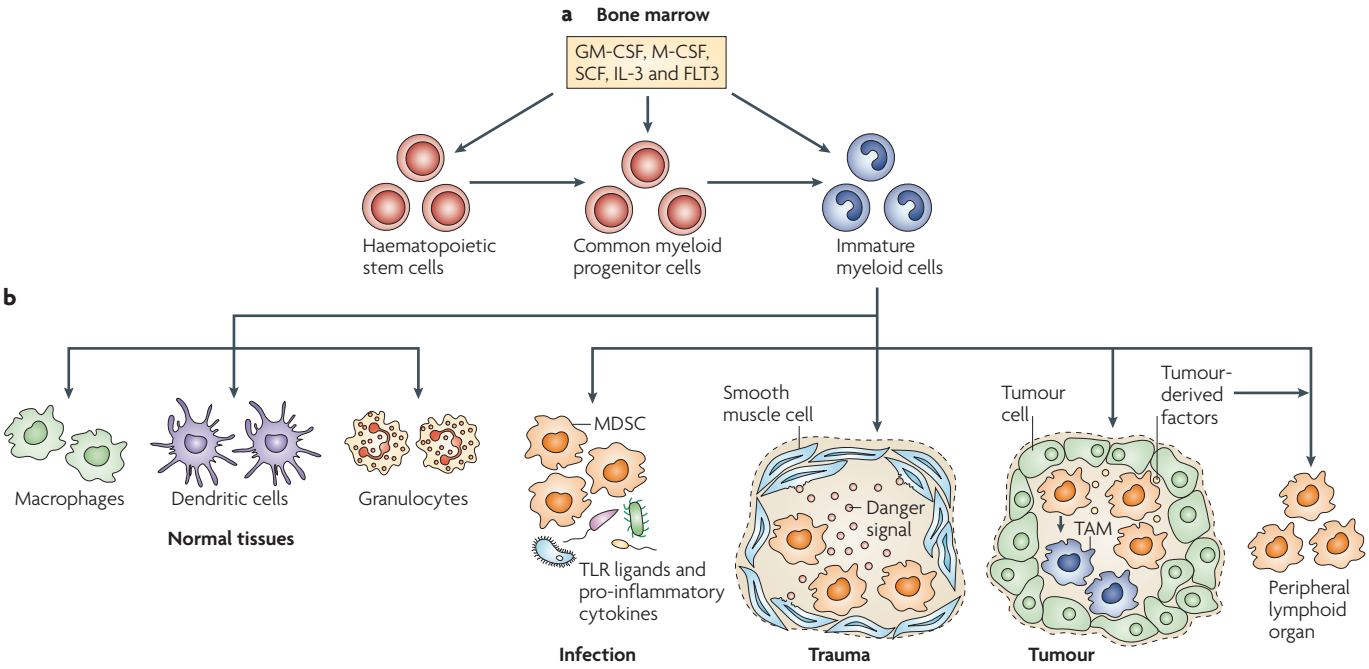

Figure 1 | **The origin of MDSCs. a** | Immature myeloid cells (IMCs) are part of the normal process of myelopoiesis, which takes place in the bone marrow and is controlled by a complex network of soluble factors, including cytokines (such as granulocyte/macrophage colony-stimulating factor (GM-CSF), macrophage CSF (M-CSF), stem-cell factor (SCF), interleukin-3 (IL-3) and FMS-related tyrosine kinase 3 (FLT3)) and cell-expressed molecules (such as Notch; not shown). Haematopoietic stem cells differentiate into common myeloid progenitor cells and then into IMCs. **b** | Normally, IMCs migrate to different peripheral organs, where they differentiate into macrophages, dendritic cells or granulocytes. However, factors that are produced during acute or chronic infections, trauma or sepsis, and in the tumour microenvironment 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 also accumulate in peripheral lymphoid organs in response to tumour-derived factors. They can also differentiate into tumour-associated macrophages (TAMs) within the tumour microenvironment; the phenotype and function of TAMs is distinct from that of MDSCs. TLR, Toll-like receptor.

together with CFA, recombinant vaccinia virus expressing IL-2, or staphylococcal enterotoxin A[8,37,38]. Based on these observations, it is clear that an immunosuppressive MDSC population frequently expands in many pathological conditions.

Expansion and activation of MDSCs

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

Mechanisms of MDSC expansion. Factors that induce MDSC expansion can include cyclooxygenase 2 (also known as PTGS2), prostaglandins[39–41], stem-cell factor (SCF)[39], M-CSF, IL-6 (REF. 42), granulocyte/macrophage CSF (GM-CSF)[41] and vascular endothelial growth factor (VEGF)[43] (TABLE 1). Most of these factors trigger signalling pathways in MDSCs that converge on Janus kinase (JAK) protein family members and signal transducer and activator of transcription 3 (STAT3) (FIG. 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 the supernatant from tumour-cell cultures resulted in the activation of JAK2 and STAT3, and was associated with an expansion of MDSCs *in vitro*. However, this expansion was abrogated when STAT3 expression in haematopoietic progenitor cells was inhibited[46]. Moreover, ablation of STAT3 expression through the use of 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 the upregulation of the expression of B-cell lymphoma XL, cyclin D1, MYC and survivin. So, abnormal and persistent activation of STAT3 in myeloid progenitor cells prevents their differentiation into mature myeloid cells and thereby promotes MDSC expansion.

Myelopoiesis
The process of differentiation of common myeloid progenitor cells to polymorphonuclear leukocytes and monocytes.

Table 1 | **Factors implicated in the expansion and activation of MDSCs in cancer**

Factor	Tumour model (mice)	Type of cancer (humans)	Refs
VEGF	Breast cancer, sarcoma, melanoma, lymphoma and lung carcinoma	Breast cancer, renal-cell cancer and pancreatic cancer	43,100, 108–112
GM-CSF	Lewis lung carcinoma, colon carcinoma, mammary adenocarcinoma and Ts/a tumour	Melanoma	41,113–118
G-CSF	Lewis lung carcinoma, methA sarcoma and melanoma	ND	119
M-CSF	Sarcoma and mammary carcinoma	Human renal carcinoma cell lines	96,120
Gangliosides	Neuroblastoma and glioma	ND	121,122
Prostaglandins	Mammary carcinoma, lung cancer, renal cancer and colon cancer	ND	40,61,102
IFNγ	Mammary adenocarcinoma, fibrosarcoma, colon carcinoma and lymphoma	ND	20,123–125
C5a	Cervical cancer and lung cancer	ND	126
SCF	Colon carcinoma	ND	39
S100A8 and S100A9	Colon carcinoma, lymphoma, fibrosarcoma and mammary carcinoma	ND	49,50
TGFβ	Colon carcinoma, fibrosarcoma and mammary adenocarcinoma	Head and neck cancer	57,127–129
IL-1β	Fibrosarcoma and mammary carcinoma	ND	130,131
IL-6	Mammary carcinoma	ND	42
IL-10	Colon cancer, melanoma and mammary carcinoma	ND	19,40
IL-12	Colon cancer	ND	9
IL-13	Colon carcinoma, fibrosarcoma, mammary adenocarcinoma and lymphoma	ND	20,57
MMP9	Colon carcinoma, Lewis lung carcinoma and mammary carcinoma	ND	101,132
CCL2	Lewis lung carcinoma, methA sarcoma, melanoma and lymphoma	ND	17,119, 125,133
CXCL5 and CXCL12	Mammary adenocarcinoma	ND	127

C5a, complement component 5a; CCL2, CC-chemokine ligand 2; CXCL, CXC-chemokine ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage CSF; IL, interleukin; IFNγ, interferon-γ; M-CSF, macrophage CSF; MDSCs, myeloid-derived suppressor cells; MMP9, matrix metalloproteinase 9; ND, not determined; S100A, S100 calcium-binding protein A; SCF, stem cell factor; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

Recent findings suggest that STAT3 also regulates MDSC expansion by inducing the expression of S100 calcium-binding protein A8 (S100A8) and S100A9, the receptors for which are also expressed on the cell surface of MDSCs. 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 tumour-bearing and naive transgenic mice that over-express S100A9. By contrast, MDSCs did not expand in the peripheral blood and spleens of mice that were 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 site of the tumour by binding to carboxylated N-glycan receptors, which are expressed on the surface of MDSCs[50]. Blocking the binding of S100A8 and S100A9 to their receptors on MDSCs *in vivo* with a carboxylated-N-glycan-specific antibody reduced the number of MDSCs 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 these regions of dysplasia and adenoma. Furthermore, administration of a carboxylated-N-glycan-specific monoclonal antibody (mAbGB3.1) was found to markedly reduce chronic inflammation and tumorigenesis[51]. Although more work is required to investigate the mechanisms that are involved in this process, these studies suggest that S100A8 and/or S100A9 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 also factors that induce MDSC activation. The expression of these factors, which are produced mainly by activated

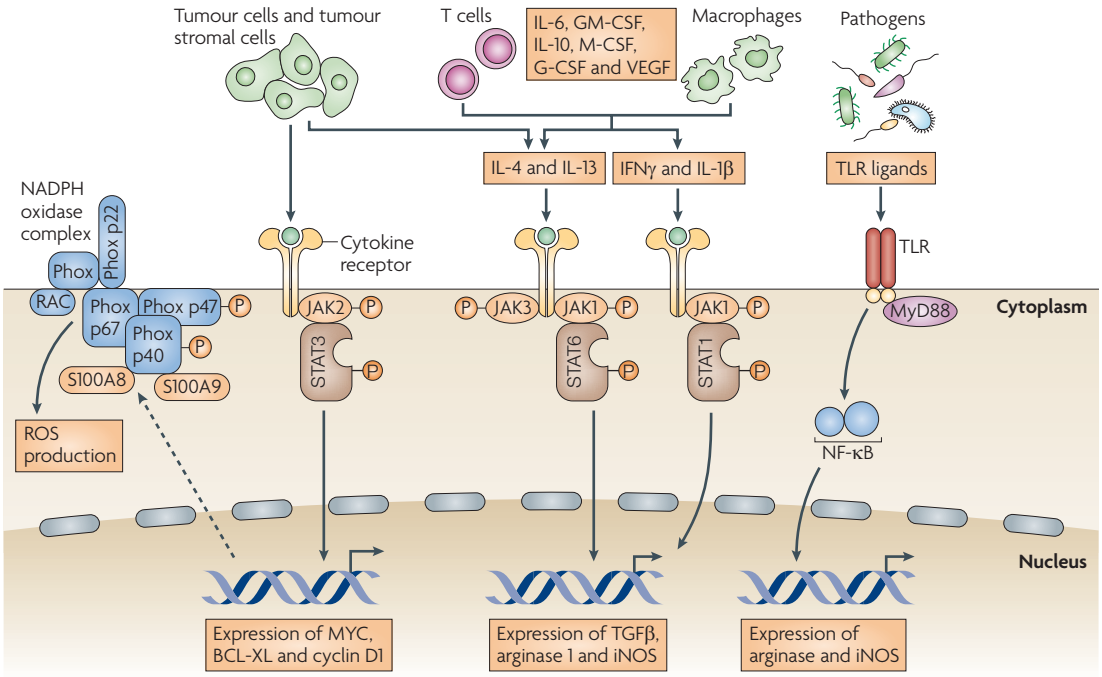

Figure 2 | **Signalling pathways involved in the expansion of MDSCs.** The accumulation of myeloid-derived suppressor cells (MDSCs) is regulated by several factors that are released by tumour cells, tumour stromal cells, activated T cells and macrophages, apoptotic tumour cells, bacterial and viral agents, and by pathogen-infected cells. These factors trigger several different signalling pathways in MDSCs that mainly involve the signal transducer and activator of transcription (STAT) family of transcription factors. STAT3 regulates the expansion of MDSCs by stimulating myelopoiesis and inhibiting myeloid-cell differentiation, and it promotes MDSC survival by inducing the expression of MYC, B-cell lymphoma XL (BCL-XL) and cyclin D1. It also contributes to the increased production of reactive oxygen species (ROS) by MDSCs. The activation of STAT6 and STAT1, as well as Toll-like receptor (TLR)-mediated activation of nuclear factor-κB (NF-κB), by these factors results in the activation of MDSCs, which leads to the upregulation of arginase 1 and inducible nitric oxide synthase (iNOS), and to the increased production of suppressive cytokines, such as transforming growth factor-β (TGFβ). In combination with STAT3, STAT1 and STAT6 also contribute to upregulation of ROS production by MDSCs (not shown). S100 calcium-binding protein A8 (S100A8) and S100A9 directly bind to components of NADPH oxidase complex. This binding potentiates the activation of NADPH oxidase in MDSCs, which causes increased production of ROS, thereby leading to the observed suppressive effects. It is probable that MDSC activation through TLRs has an important role during pathogenic infections. G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage CSF; IFNγ, interferon-γ; IL, interleukin; JAK, Janus kinase; M-CSF, macrophage CSF; MyD88, myeloid differentiation primary-response gene 88; Phox, phagocyte oxidase; VEGF, vascular endothelial growth factor.

T cells and tumour stromal cells, is induced by different bacterial and viral products, or as a result of tumour-cell death[26]. These factors, which include IFNγ, ligands for Toll-like receptors (TLRs), IL-4, IL-13 and transforming growth factor-β (TGFβ), activate several different signalling pathways in MDSCs that involve STAT6, STAT1 and nuclear factor-κB (FIG. 2).

Blockade of IFNγ, which is produced by activated T cells, abolishes MDSC-mediated T-cell suppression[17,52]. STAT1 is the main transcription factor that is activated by IFNγ-mediated signalling and is involved in the upregulation of arginase 1 and iNOS expression by MDSCs in the tumour microenvironment. Indeed, MDSCs from *Stat1*$^{-/-}$ mice failed to upregulate the expression of arginase 1 and iNOS and therefore did not inhibit T-cell responses[53]. Consistent with other findings, IFNγ that was produced by activated T cells and MDSCs induced the expression of iNOS and synergized with the activity of IL-4Rα and arginase 1 (REF. 20), both of which have been implicated in the suppressive function of MDSCs[20].

An important role for the signalling pathway downstream of 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 indicated by several studies. Activation of the IL-4Rα signalling pathway in freshly isolated MDSCs or cloned MDSC lines by IL-4 induced the expression of arginase 1 (REF. 54). In addition, IL-4 and IL-13 were found to upregulate the activity of arginase 1, 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 arginase 1 by MDSCs[56]. Moreover, the IL-4Rα–STAT6 pathway was also found to be involved in the IL-13-induced production of TGFβ by MDSCs in mice with sarcoma, which resulted in decreased tumour immunosurveillance[57]; this could be regulated by neutralizing both TGFβ and IL-13 (REF. 57). However, in a model of breast cancer, mice that were deficient in IL-4Rα retained high numbers of MDSCs in the spleen after the

Tumour immunosurveillance
The process of recognition of tumour antigens and elimination of the tumours by the immune system.

surgical removal of mammary tumours[56]. In a different study that evaluated the role of TGFβ (but not the role of IL-4Rα), a TGFβ-specific blocking antibody failed to reverse T-cell anergy in B-cell lymphoma *in vitro*[58]. Collectively, these data indicate 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 that is 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. This MDSC expansion 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 population during polymicrobial sepsis. This 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 conditions of sepsis[26]. These observations indicate that the activation of MDSCs is a basic outcome of the host innate immune response to pathogens that express TLR ligands.

It is important to note that an increase in the generation and/or recruitment of IMCs in the context of acute infectious diseases or following vaccination does not necessarily reflect an expansion of an immunosuppressive MDSC population. It is probable that under pathological conditions the expansion of a suppressive MDSC population is regulated by two different groups of factors that have partially overlapping activities: those that induce MDSC expansion and those that induce their activation. 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 activities of MDSCs require direct cell–cell contact, which suggests that they function either through cell-surface receptors and/or through the release of short-lived soluble mediators. The following sections describe the mechanisms that have been implicated in MDSC-mediated suppression of T-cell function.

Arginase 1 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 1 (which converts L-arginine to urea and L-ornithine). MDSCs express high levels of both arginase 1 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 L-arginine and the regulation of T-cell proliferation[11,61]. The increased activity of arginase 1 in 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 expression of CD3 ζ-chain[62] and preventing their upregulation of the expression of the cell cycle regulators cyclin D3 and cyclin-dependent kinase 4 (REF. 63). NO suppresses T-cell function through various different mechanisms that involve the inhibition of JAK3 and STAT5 function 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 from both tumour-bearing mice and patients with cancer[6,10,13,53,67–70]. Inhibition of ROS production by MDSCs isolated from tumour-bearing mice and patients with cancer completely abrogated the suppressive effect of these cells *in vitro*[10,13,67]. Interestingly, the ligation of integrins that are 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-3, IL-6, IL-10, platelet-derived growth factor and GM-CSF, can induce the production of ROS by MDSCs (for a review, see REF. 71).

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

Peroxynitrite. More recently, it has emerged that peroxynitrite is a crucial mediator of MDSC-mediated suppression of T-cell function. Peroxynitrite is a product of a chemical reaction between NO and superoxide anion, and is one of the most powerful oxidants that are produced in the body. It induces the nitration and nitrosylation of the amino acids cysteine, methionine, tryptophan and tyrosine[72]. Increased levels of peroxynitrite are present at sites in which MDSCs and inflammatory cells accumulate, 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], an effect that has been linked with T-cell unresponsiveness**.** One study[79] 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 that peroxynitrite was produced in the tumour microenvironment. Inhibiting the activity of arginase 1 and iNOS, which are expressed in malignant but not in normal prostate tissue, led to decreased tyrosine

T-cell anergy
A state of T-cell unresponsiveness to stimulation with antigen. It can be induced by stimulation with a large amount of specific antigen in the absence of the engagement of co-stimulatory molecules.

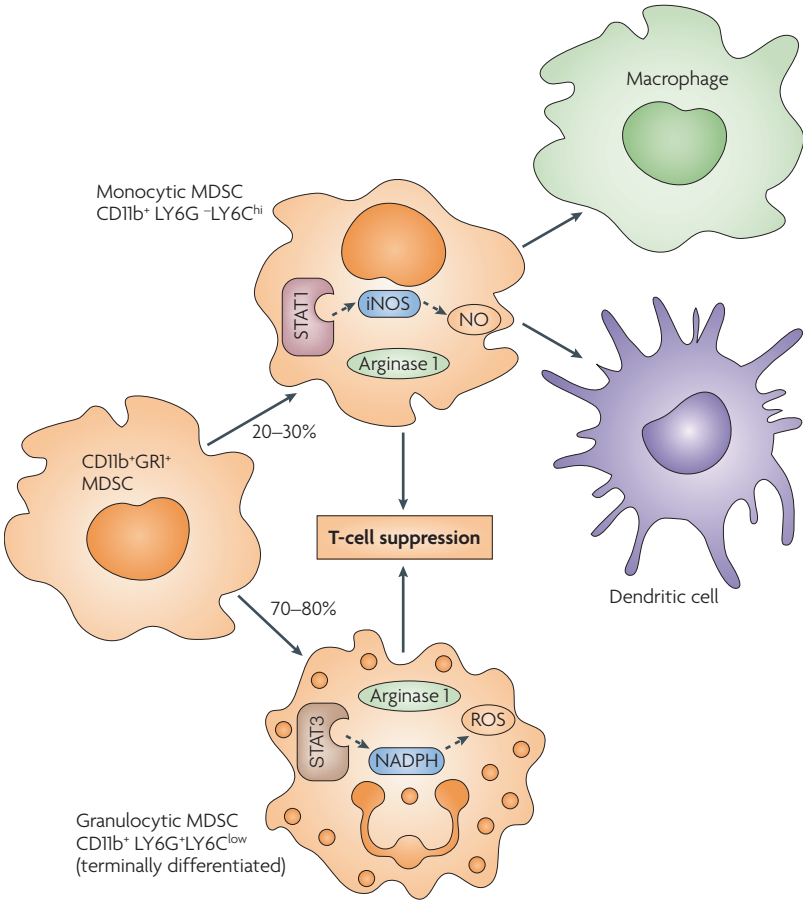

Figure 3 | **Suppressive mechanisms mediated by different subsets of MDSCs.** Myeloid-derived suppressor cells (MDSCs) consist of two main subsets: monocytic MDSCs, which have a $CD11b^+LY6G^-LY6C^{hi}$ phenotype, and granulocytic MDSCs, which have a $CD11b^+LY6G^+LY6C^{low}$ phenotype. In most tumour models, it is predominantly (70–80%) the granulocytic subset of MDSCs that expands. We suggest that the granulocytic subset of MDSCs has increased activity of signal transducer and activator of transcription 3 (STAT3) and NADPH, which results in high levels of reactive oxygen species (ROS) but low nitric oxide (NO) production. ROS and, in particular, peroxynitrite (the product of a chemical reaction between superoxide anion and NO) induces the 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 of NO but low ROS production. NO, which is produced by the metabolism of L-arginine by iNOS, suppresses T-cell function through various different mechanisms that involve the inhibition of Janus kinase 3 and STAT5, the inhibition of MHC class II expression and the induction of T-cell apoptosis. Both subsets have increased levels of arginase 1, which causes T-cell suppression through depletion of L-arginine. Only monocytic MDSCs can differentiate into mature dendritic cells and macrophages *in vitro*.

Regulatory T (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 the expression of CD25 and the transcription factor forkhead box P3.

nitration and restoration of T-cell responsiveness to tumour antigens. In addition, we have shown that peroxynitrite production by MDSCs during direct contact with T cells resulted in nitration of the T-cell receptor and CD8 molecules, which altered the specific peptide binding of the T cells and rendered them unresponsive to antigen-specific stimulation[80]. However, the T cells maintained their responsiveness to non-specific stimuli. This phenomenon of MDSC-mediated 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 MDSCs might use different mechanisms 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 MDSCs 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; both subsets expressed arginase 1 (REF. 6) (FIG. 3). Interestingly, both MDSC subsets suppressed antigen-specific T-cell proliferation to an equal extent despite having different mechanisms of action. Consistent with these observations, another study[17] reported two distinct MDSC subsets in tumour-bearing mice, one that consisted of mononuclear cells resembling 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 they used distinct effector molecules and signalling pathways. The suppressive activity of the granulocytic subset depended on arginase 1, whereas that of the monocytic subset depended on STAT1 and iNOS[17]. Finally, the same trend was observed during *T. cruzi* infection: monocytic MDSCs produced NO and strongly inhibited T-cell proliferation, whereas granulocytic MDSCs produced low levels of NO[15]. However, this subset did not inhibit T-cell proliferation, although it did produce superoxide anion[15]. The biological significance of such functional dichotomy between two MDSC subsets remains to be elucidated.

Induction of T_{Reg} cells. Recently, the ability of MDSCs to promote the *de novo* development of forkhead box P3 (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 the production of NO[19]. In mice bearing 1D8 ovarian tumours, the MDSC-mediated induction of T_{Reg} cells required the expression of cytotoxic lymphocyte antigen 4 (CTLA4; 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 involved arginase 1 and the capture, processing and presentation of tumour-associated antigens by MDSCs, but was independent of TGFβ[58]. By contrast, another group[17] 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 induction. Furthermore, in a rat model of kidney allograft tolerance that was induced using 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 through direct cell–cell interactions. Furthermore, MDSCs and T_{Reg} cells might be linked in a common immunoregulatory network (see later).

Tissue-specific effects of MDSCs

A fundamental unresolved question in this field is whether MDSCs mediate antigen-specific or antigen non-specific suppression of T-cell responses. Provided that MDSCs and T cells are in close proximity, the factors that mediate the suppressive function of MDSCs (ROS, arginase 1 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-non-specific 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. This hypothesis 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 antigen-non-specific interactions[82,84,85]. Such stable contacts are necessary for MDSC-derived ROS and peroxynitrite to mediate their effects on the molecules expressed 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 does it prevent antigen-non-specific T-cell activation. Other evidence that supports the hypothesis that MDSCs mediate antigen-specific T-cell 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 an MHC class I-specific antibody abrogated MDSC-mediated inhibition of $CD8^+$ T-cell responses *in vitro*[86]. The MHC class I-restricted nature of MDSC-mediated $CD8^+$ T-cell suppression has also been shown *in vivo* in tumour models[53] and in a 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 patients with cancer 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 suggests that the nature of MDSC-mediated suppression at the site of the tumour is different to that which occurs in the periphery. MDSCs actively migrate to the site of the tumour[10], where they upregulate the expression of arginase 1 and iNOS, downregulate the production of ROS and/or rapidly differentiate into tumour-associated macrophages (TAMs)[52]. The levels of NO and arginase 1 that are produced by tumour-associated MDSCs and TAMs are much higher than those produced by MDSCs that are 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 an antigen-non-specific manner (FIG. 4). The mechanisms by which MDSC functions are regulated within the tumour microenvironment, and how they differ from those that operate in the periphery, remain unclear. It is possible that tumour stromal cells, hypoxia and/or the acidophilic environment have a role in the regulation of MDSC function.

Tumour-associated macrophage
A cell that differentiates from circulating blood monocytes and myeloid-derived suppressor cells that have infiltrated tumours. These cells can have positive or negative effects on tumorigenesis.

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 induce an immune response has resulted in a paradigm shift regarding 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 tumour-bearing hosts, it is probable that the same strategies will be useful in other pathological conditions in which the therapeutic aim is inhibition or elimination of MDSCs.

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 functions. 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 progenitor cells 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, administration of therapeutic concentrations of all-*trans* retinoic acid (ATRA) results in a substantial decrease in the numbers of MDSCs in patients with cancer and tumour-bearing mice. ATRA has been shown to induce the differentiation of MDSCs into DCs and macrophages *in vitro* and *in vivo*[12,86,96]. It is probable that ATRA preferentially induces the differentiation of the monocytic subset of MDSCs and mediates the apoptosis of the granulocytic subset. The main mechanism of ATRA-mediated differentiation involves an upregulation of glutathione synthesis and a reduction in ROS levels in MDSCs[97]. In studies using tumour-bearing mice, decreasing the number of MDSCs resulted in increased tumour-specific T-cell responses, and the combination of ATRA and two different types of cancer vaccine prolonged

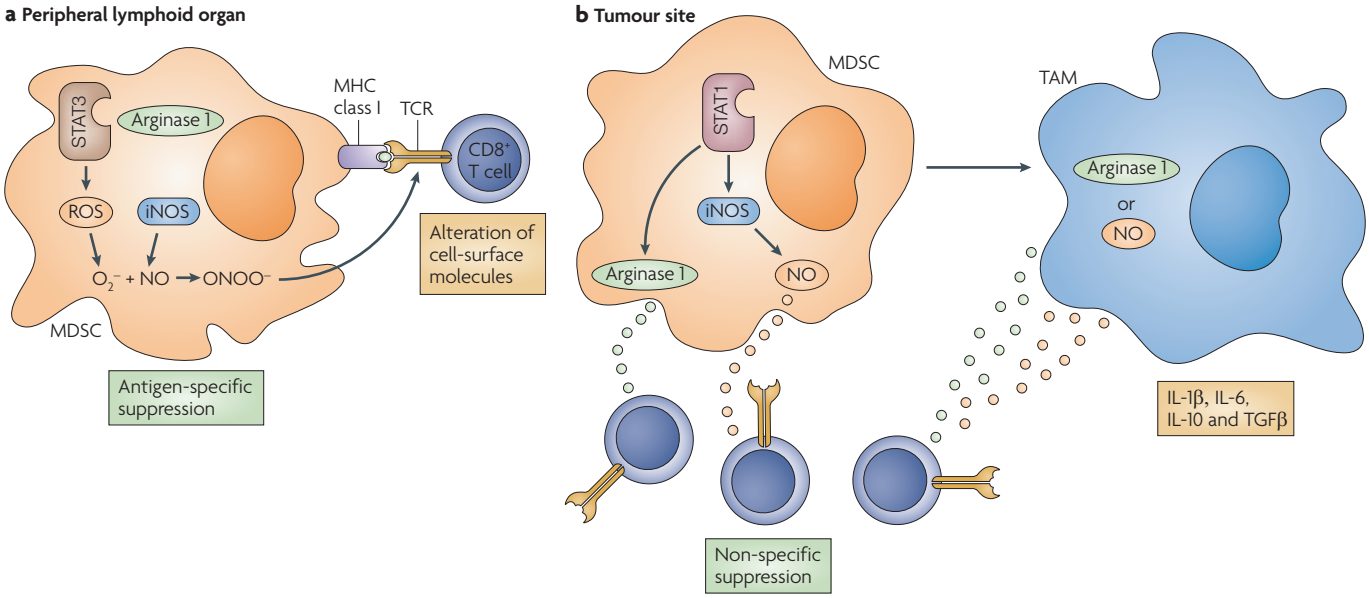

Figure 4 | **The mechanisms of MDSC-mediated immune suppression differ in peripheral lymphoid organs and at the site of a tumour.** Myeloid-derived suppressor cells (MDSCs) migrate to tumour sites and peripheral lymphoid organs. **a** | In peripheral lymphoid organs, MDSCs produce high levels of reactive oxygen species (ROS), including peroxynitrite ($ONOO^-$), and upregulate signal transducer and activator of transcription 3 (STAT3) activity. This is associated with a moderate increase in arginase 1 activity and low levels of nitric oxide (NO) production. MDSCs can 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 on the T-cell receptor (TCR) and CD8 molecules on the surface of T cells, which causes the T cells to become unresponsive to antigen-specific stimulation. However, these cells retain the ability to respond to antigen-non-specific stimulation with CD3- and CD28-specific antibodies. **b** | By contrast, MDSCs that migrate to the site of the tumour upregulate STAT1 activity, produce high levels of inducible nitric oxide synthase (iNOS), NO and arginase 1; this is associated with low levels of ROS. The high levels of arginase 1 and NO that are released by MDSCs inhibit $CD8^+$ T-cell function in a non-specific manner. MDSCs at the tumour site can also differentiate into tumour-associated macrophages (TAMs). In contrast to MDSCs, TAMs upregulate the expression of either arginase 1 or iNOS, depending on the nature of the tumour microenvironment (see REF. 91), but not of both proteins. TAMs acquire the ability to produce several suppressive cytokines, such as interleukin-1β (IL-1β), IL-6, IL-10 and transforming growth factor-β (TGFβ). Together with MDSCs, TAMs contribute to antigen-non-specific T-cell suppression in the tumour microenvironment.

the anti-tumour effect of the vaccine 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 may lead to the identification of other agents that have a similar effect. So far, evidence suggests that vitamin D3 may be another compound that has 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 the interaction with its receptor, KIT, decreased MDSC expansion and tumour angiogenesis[39]. VEGF, another tumour-derived factor that is involved in promoting MDSC expansion, might also be a useful target by which to manipulate MDSCs. 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 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 the $CD11b^+VEGFR1^+$ population of MDSCs in the peripheral blood[100]. Whether avastatin treatment resulted in an improvement in antitumour responses in these patients has not been determined. Finally, inhibition of the function of matrix metalloproteinase 9 in tumour-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

Table 2 | **Therapeutic strategies to target MDSCs**

Therapeutic agents	Type of cancer tested	Refs
Vitamin D3	Head and neck cancer*	98
All-*trans* retinoic acid	Sarcoma‡, colon carcinoma‡ and metastatic renal-cell carcinoma*	96,21
KIT-specific antibody	Colon carcinoma‡	39
VEGF–trap§	Solid tumours*	89
VEGF-specific antibody (avastin)	Metastatic renal-cell cancer*	100
Cyclooxygenase 2 inhibitor (SC58236)	Mammary carcinoma‡	40
Bisphosphanate, sildenafil and tadalafil	Mammary carcinoma, colon carcinoma and fibrosarcoma‡	104
Amino-bisphosphonate	Mammary tumours‡	101
Nitroaspirin	Colon carcinoma‡	105
Gemcitabine	Lung cancer, mammary tumours‡	106, 107

*Agents were tested in humans. ‡Agents were tested in mice. §VEGF-trap is a fusion protein that binds all forms of VEGF and placental growth factor. VEGF, vascular endothelial growth factor.

be achieved is cyclooxygenase 2. Cyclooxygenase 2 is required for the production of prostaglandin E2, which has been shown to induce the upregulation of arginase 1 expression by MDSCs (and thereby induce their suppresive function) in 3LL tumour cells[61] and mammary carcinoma[40]. Accordingly, cyclooxygenase 2 inhibitors were found to downregulate the expression of arginase 1 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 1 and iNOS by MDSCs, thereby inhibiting their suppressive function in growing tumours[104]. This resulted in the induction of a measurable antitumour 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 arginase 1 and iNOS in spleen MDSCs[105]. In addition, nitroaspirin inhibited the function of MDSCs and increased the number and function of tumour-antigen-specific T cells when administered in conjunction with endogenous retroviral gp70 antigen[105].

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

Evidence suggests that there is a broad range of methods that could be effective for targeting the number and/or function of MDSCs *in vivo*. These strategies will undoubtedly help to further investigate the biology of these cells and to advance clinical applications for the treatment of 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 and are not immunosuppressive when they are 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 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 the expansion of T_{Reg} cells. Future studies will reveal whether MDSCs can be considered part of a natural immune regulatory network.

Concluding remarks

Research into the field of MDSC biology has yielded more questions than answers. The roles of specific MDSC subsets in mediating T-cell suppression, and the molecular mechanisms that are responsible for the 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 induce 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 importance. Furthermore, 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 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 many more discoveries that further our understanding of the biology and clinical use of MDSCs.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ARG1 | CD11b | CD115 | CD124 | JAK2 | LY6C | LY6G | S100A8 | S100A9 | STAT3 | TGFβ | VEGF

FURTHER INFORMATION

Dmitry I. Gabrilovich's homepage: http://www.moffitt.org/Site.aspx?spid=1E75B4346484453782CBE2BEE945D9BF&SearchType=Researcher

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