Myeloid-derived suppressor cells and their “inconvenient” plasticity

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ABSTRACT

Myeloid-derived suppressor cells (MDSCs) comprise certain types of myeloid subsets with strong immunosuppressive activities, which expand at high levels in pathological conditions such as cancer. A major drawback in the study of MDSCs is the extraordinary plasticity of the myeloid lineage that hampers the identification of MDSC subsets, especially in humans. Here we provide a brief overview on MDSCs, their differentiation and the current difficulties in classifying these immunosuppressive subsets.

Myeloid-derived suppressor cell subsets

Myeloid cells comprise a highly diverse population involved in the maintenance of the equilibrium of our immune system. They undergo major phenotypic changes (activation) as a result of engaging with pathogen- or danger-associated stimuli (pathogen associated molecular pattern (PAMPs) and danger associated molecular pattern (DAMPs). Once the source of stimuli disappear activation terminates. However, this is not the case of inflammatory chronic diseases or cancer, disorders that usually maintain continuous inflammatory stimuli and sustained exposure to different antigens. In these conditions, myeloid reprogramming takes place, leading to the differentiation of myeloid cells with different characteristics as those found in non-pathological conditions. Both in murine cancer models and in humans with neoplastic disorders, tumors disrupt the whole systemic homeostasis by producing a wide collection of cytokines, growth factors and chemokines that are distributed through blood and lymph1,2. These cytokines reach the bone marrow and significantly perturb the physiological myelopoiesis, especially when the tumor burden is high. Amongst these soluble mediators, tumors usually produce high levels of GM-CSF, M-CSF, IL4, IL6, IL18, IL13, prostaglandins, IL10 and TGF-β3-9. High levels of GM-CSF are critical for the differentiation of MDSCs instead of myeloid cells such as dendritic cells, monocytes, granulocytes or macrophages10,11.

The first reports on the accumulation of myeloid cells within tumors as a sign of poor prognosis were published in the early 1970s12. Since then numerous studies have clearly demonstrated that systemic expansion and accumulation of myeloid populations with suppressive activity is associated with tumor progression and metastasis, both in preclinical and clinical cancer models. Then, in 2007 it was proposed that myeloid cells with suppressive activities should be covered by a common denomination; myeloid...
derived suppressor cells (MDSC). This was an attempt to acknowledge the existence of these cells as distinct entities from those found in non-pathological conditions.

Since the 1990s, numerous studies and extensive work in this subject uncovered two distinct MDSC subsets, well characterized in murine models: monocytic (M)-MDSC present a distinct phenotype which reminds to that of inflammatory monocytes (CD11b+Ly6G−Ly6C(lo)) while granulocytic (G) or polymorphonuclear (PMN)-MDSC show a phenotype closely related to granulocytes (CD11b+Ly6G+Ly6C(lo)). It has to be remarked that these two phenotypes are nearly exactly the same as found in non-pathological counterparts, and the truly differential characteristic is the high suppressive activities of MDSCs towards other immune cell types such as activated T cells.\(^1\)

The acquisition of strong immunosuppressive activities is the probable result of major transcriptomic and metabolic changes, which in turn are regulated by a distinct core of the kinases including ERK, AKT and PKCs (Figure 1)\(^{13,15}\). Firstly, MDSCs are frequently phenotypically “immature”, meaning that they express on the surface low levels of major histocompatibility molecules (MHC) and some (but not all) co-stimulatory molecules\(^{11,16}\). This absence of MHC molecules on their surface prevents efficacious antigen presentation to T cells. However, MDSCs do inhibit previously-activated T cells through a variety of mechanisms (Figure 2), which include depletion of essential aminoacids such as glutamine, production of immunosuppressive cytokines such as TGF-β or IL-10 \(^{11,17}\), expression of T cell inhibitory molecules such as PD-L1\(^{13,18}\) and the production of nitric oxide (NO) by constitutive expression of iNOS. NO production causes the nitrosylation of a high number of intracellular proteins, a post-translational modification that alters their activities\(^{11,19}\).

Nevertheless, the presence of specific surface markers made feasible their purification from murine models and their characterization by genomic\(^{20}\) and proteomic approaches\(^{13,14}\). In vitro differentiation systems and also some in vivo studies in murine cancer models allowed the demonstration that granulocytic MDSCs comprise the final stage of monocytic MDSC maturation\(^{21,11}\).

Even though there is certainly a lack of specific markers exclusively owned only by MDSC populations, a variety of panels of markers characterising human MDSC monocytic and granulocytic populations have been published. Indeed, this has been probably the result of trying to characterize the phenotype of human MDSC counterparts. While the phenotype of G-MDSC and M-MDSC in mice is certainly straightforward (albeit of their similarities with non-pathological myeloid subsets), this is not the case in humans. Early on, human monocytic MDSC were defined as CD11b+CD14+HLA-DR−/lo CD15+ marker of G-MDSC\(^{24}\). Again, in resemblance of the murine model with G-MDSCs resembling granulocytes. Nevertheless, the lookout for novel MDSC-specific markers has not ceased, and recently LOX1 was described as specific marker of G-MDSC\(^{24}\).

As commented above, the particularity of MDSC resides on their strong suppressive activities, which are absent in their non-pathological counterparts. Since the identification of their phenotypical markers, these cells have been isolated or produced in vitro, and numerous studies have been characterizing their functional activities. These studies have uncovered a wide range of suppressive mechanisms that MDSCs employ to suppress the effector activities and proliferation of different immune cell populations, most extensively in activated T cells. Many of these mechanisms are shared by previously-known immunosuppressive pathways exerted by, for example, M2 macrophages or even regulatory T cells (Tregs).

In recent years there is a growing body of literature unveiling the role of MDSCs in non-neoplastic pathological conditions such as autoimmune and many infectious diseases, graft versus host response or in maternal-foetal tolerance (reviewed elsewhere\(^{1,25}\)).

**Myeloid plasticity as the main “inconvenience”**

The situation nowadays on MDSCs is certainly one of confusion\(^2\). By no means, many immunologists and
researchers do not accept their existence as specific myeloid entities different from the classical myeloid cell types. And even those who acknowledge their existence do not agree in many things, including their phenotype, origins and relationships amongst these cell types. Why is that? Indeed, the culprit here is the extraordinary differentiation plasticity of the myeloid lineage both in murine models and humans. This is the main "inconvenience". This "inconvenient" situation also occurred some time ago with Tregs, which also lacked specific markers of their...
own during the 70s and 80s\textsuperscript{26}. Only when Tregs were defined as CD25\textsuperscript{high} Foxp3 positive, they could be studied, characterized, and more importantly, accepted by the scientific and medical community.

This is certainly not the case yet with MDSCs. The immense plasticity of myeloid populations from the bone marrow to peripheral tissues subjected under the enormous pressure of tumor-derived factors makes it difficult to clearly discriminate between MDSCs, neutrophils, monocytes or tolerogenic dendritic cells. As a consequence, some authors have proposed the classification of all myeloid populations with suppressive activity under the common denomination of myeloid regulatory cells\textsuperscript{27,28}. But again, it is unclear whether this common denominator is yet another way to not addressing the main problem with MDSCs. Their enormous plasticity.

### Myeloid plasticity in pathological situations

Confusion due to the remarkable plasticity of myeloid cells starts with the MDSCs themselves in the murine system. Since their identification and phenotypic characterization, monocytic and granulocytic MDSCs have been considered to be lineages apart rather than belonging to the same “cell type” under distinct differentiation stages\textsuperscript{9,29}. This distinction has been maintained through most of the published works, and remains to be so nowadays. This is likely caused by their significant phenotypic resemblance to monocytes and granulocytes in non-pathological conditions. Therefore, it was only logical to assume that monocytic MDSCs came from the same differentiation route than monocytes and DCs\textsuperscript{30}, while granulocytic MDSCs came from the differentiation pathway of granulocytes\textsuperscript{31}. This distinction is of relevance, as differentiation of monocyctic and granulocytic lineages takes place early in myelopoiesis at the granulocyte-monocyte progenitor (GMP) stage. Expression at this stage of PU.1 and IRF8 transcription factors leads GMP towards the monocytic lineage differentiation, which includes DCs, monocytes and macrophages\textsuperscript{32-34}. In contrast, expression of c/EBPα expression causes GMP to differentiate to the granulocytic lineage\textsuperscript{35,36}. However, MDSC differentiation cannot be compared to physiological myelopoiesis, as it takes place under specific circumstances. Differentiation in neoplastic conditions resembles much more myelopoiesis under stress, which favors fast expansion of granulocytes through an alternative non-steady state differentiation route regulated by c/EBPβ\textsuperscript{37,38}. Indeed, at least in murine models it seems that granulocytic MDSCs are the terminal differentiation stage of monocytic MDSC subsets. This was demonstrated in vivo in tumor-bearing mice, in which G-MDSCs quickly differentiated from M-MDSCs by epigenetic silencing of retiblastoma expression, possibly regulated by hypoxia\textsuperscript{39}. The demonstration that this was indeed the case came from in vitro differentiation of MDSCs from murine bone marrow. Purified monocytic MDSCs quickly converted to granulocytic MDSCs by strong up-regulation of Ly6G\textsuperscript{11}, which in turn could be regulated by the interplay of distinct signalling pathways regulating different aspects of MDSC functions\textsuperscript{13}. A key question remains whether the same differentiation pathways are present in the human system. At least in the murine system, it has been proposed that MDSCs arise probably from a CD11b+ Ly6Cneg Ly6Gneg GMP-like or MDP-like precursor that will differentiate into M-MDSC. Then, following the activity of the c/EBPβ transcription factor, M-MDSC will quickly differentiate into G-MDSC as early as in the bone marrow or within the tumor environment through the activity of IRF8\textsuperscript{40-43}.

A strong drawback of studying MDSC differentiation in humans is the lack of efficacious in vitro differentiation systems. In most cases, “MDSCs” or rather "myeloid populations” are monitored in peripheral blood, or MDSCs differentiated from adherent monocytes obtained from peripheral blood. Therefore, it is rather hard to identify the signalling pathways regulating human MDSC differentiation when most of the times early precursors are not utilized\textsuperscript{16,44}. It goes without saying that myeloid populations infiltrating the tumor are rather heterogeneous, and sometimes it is quite challenging to identify myeloid cells as MDSCs, macrophages or tolerogenic dendritic cells\textsuperscript{16,45}.

MDSC plasticity is also evident by their intrinsic capacities of becoming immunostimulatory. Thus, intratumor MDSCs can quickly convert to immunostimulatory myeloid cells with strong T cell stimulatory activities, and turn an immunosuppressive tumor environment into an efficacious anti-tumor response. This is perfectly exemplified by the expression of IL-12, a cytokine with potent T cell stimulatory activities. Hence, modification of murine MDSCs with lentivectors expressing IL-12 can turn these cells into potent antigen-presenting cells and raise effective anti-tumor immune responses\textsuperscript{46,47}. This effect is potentiated by combining IL-12 expression with a PD-L1-silencing microRNA that enhances antigen presentation by non-professional antigen presenting cells\textsuperscript{57}, and inhibits tumor growth in vivo by delaying cancer cell proliferation and sensitizing these cells to cytotoxicity by T cells\textsuperscript{48}. IL-12 expression in several cancer models also leads to an increase of CD80, MHC class II and other maturation molecules in MDSCs, as well as an increase in myeloid DCs\textsuperscript{49,50}.

### Conclusions

It seems evident that MDSC do really exist. There is overwhelming evidence on their differentiation and their clear detrimental effects in pathologies such as cancer. However, whether MDSCs can be considered a lineage of their own is still rather hard. This is caused by the
enormous plasticity of the myeloid lineage, and the high inter-conversion capabilities of one myeloid cell type towards another depending on their environment and interactions with other cell types. Even their characteristic immunosuppressive properties can also be found (or induced) in non-MDSC counterparts. Nevertheless, it cannot be argued that MDSCs are real entities in murine models. However, human MDSCs, or rather their existence, is rather complicated to demonstrate. There is a lack of in vitro well-defined differentiation system that may allow their analysis by genomic and proteomic techniques. Indeed, most of the evidence shows an extraordinary variety of myeloid cell types in humans\textsuperscript{51}, which hampers rather than sheds light on human MDSC differentiation and their role in cancer. Nevertheless, it is likely that MDSCs do exist in humans, although their phenotypic differentiation, origin and functions may differ significantly from the murine counterparts.

Conflict of interest statement

The authors declare no conflicts of interest

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