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# Cell Lineage Commitment and Tumor Microenvironment as Determinants for Tumor-Associated Myelomonocytic Cells Plasticity

Raffaella Bonecchi[1,2], Benedetta Savino[1], Matthieu Pesant[1] and Massimo Locati[1,2]
[1]*Istituto Clinico Humanitas IRCCS, Rozzano,*
[2]*Department of Translational Medicine, University of Milan, Milan, Italy*

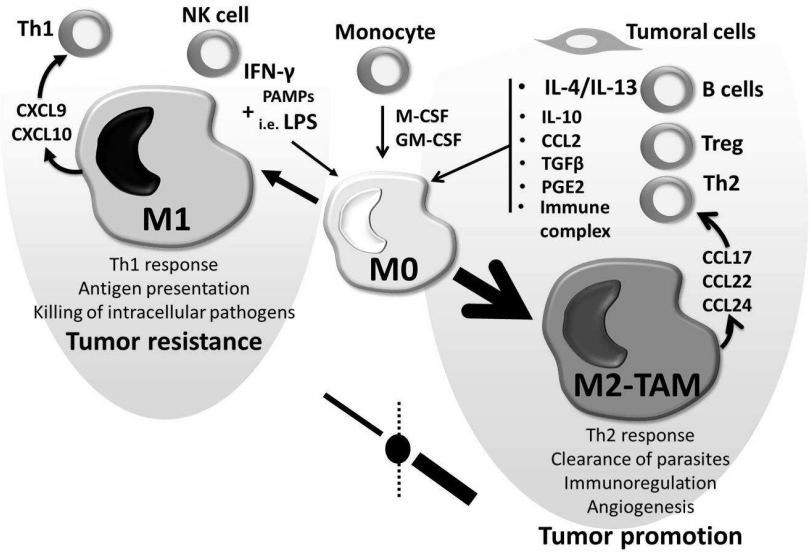
## 1. Introduction

Myelomonocytic cells have long been recognized as key elements in tumor biology, with the potential to both elicit tumor and tissue destructive reactions and to promote tumor progression. Tumor-associated macrophages (TAM) from established tumors resemble alternative-activated macrophages associated with the resolution phase of inflammatory reactions and support tumor growth, angiogenesis, tissue remodeling, metastatization, and local immunosuppression. On the other hand, myeloid-derived suppressor cells are released from bone marrow pools in tumor-bearing animals and operate immunosuppressive activities in tumor-draining lymphoid organs, thus contributing to tumor escape from immune surveillance. The ambivalent role of myelomonocytic cells in tumor biology reflects their extraordinary plasticity. Tumor-derived signals in the local microenvironment have long been recognized for their ability to dictate macrophage-polarized activation. More recently, different monocyte subsets have been identified in both human and mouse, and cell lineage commitment is now emerging as a second element dictating cell functional polarization. We will here review current knowledge on the relative contribution of these two elements in the plasticity of myelomonocytic cells in the tumor setting.

## 2. Macrophage heterogeneity and polarization mechanisms

It is well established that tumors are environments of deregulated innate and adaptive immune responses. In this scenario, several evidences link tumor initiation and progression to chronic inflammation and recent findings have started dissecting the underlying cellular and molecular mechanisms. Macrophages represent one of the major myelomonocytic-derived cell types detectable within the tumor (Condeelis & Pollard 2006; Mantovani et al. 2008). It is now widely documented that tumor-associated macrophages (TAM) infiltration and biological activities within the tumor favor tumor growth/development (Pollard 2004; Lin et al. 2006; Mantovani et al. 2008), and consistently with this TAM infiltrate is usually associated with poor prognosis (Bingle et al. 2002; Lewis & Pollard 2006; Torroella-Kouri et al. 2009; Qian & Pollard 2010).

In contrast with classically activated macrophages (also known as M1 macrophages), in most tumors macrophages present an "alternative" activation state (Elgert et al. 1998). Originally based on *in vitro* studies inspired by the Th1/Th2 paradigma, macrophage activation can be schematically reconciled to two main phenotypes (Figure 1). Macrophages stimulated with the Th1 cytokine IFNγ and bacterial components such as LPS were named classically-activated macrophages or M1. They are characterized by a high production of IL-12 and IL-23, sustain the Th1 response by producing the chemokines CXCL9 and CXCL10, exhibit cytotoxic activity and high phagocytosis capacity, a high production or reactive oxygen intermediates (ROI), and display a good antigen presentation capability (Mantovani et al. 2002; Gordon 2003; Verreck et al. 2004; Gordon & Taylor 2005; Mantovani et al. 2005; Martinez et al. 2006). As a first line of defense against pathogens M1 play an important role in protection from viral and microbial infections. By their ability to produce high amounts a pro-inflammatory cytokines and mounting an immune response they also limit tumor growth/development. At the other extreme of the macrophage polarization spectrum are cells exposed to the Th2 prototypical cytokine IL-4, referred to as alternatively-activated macrophages or M2 macrophages (Stein et al. 1992). More recently, different forms of alternative activation polarization, collectively indicated as M2-like macrophages, have been reported as a consequence to activation by other stimuli, including the combination of immune complexes and TLR ligands, IL-10, TGFβ, and glucocorticoids (Mantovani et al. 2004). In general terms, hallmarks of M2 and M2-like cells are a high expression of negative




Macrophages differentiate from monocytes after M-CSF and/or GM-CSF stimulation. Subsequent polarization pathways include classical activation induced by IFNγ and LPS (M1 macrophages) and alternative activation triggered by IL-4/IL-13 (M2 macrophages). In the tumoral microenvironment, tumor-associated macrophages (TAMs) encounter diverse polarizing stimuli produced by tumoral cells, Th2 cells, Treg cells and B cells that skew their activation state to a phenotype resembling M2 macrophages, leading to tumor promotion/development.

Fig. 1. Macrophage polarization mechanisms and cancer: a dangerous imbalance.

regulators of the inflammatory response, including IL-10 and IL-1 receptor antagonist, and scavenger and galactose-type receptors (for example CD36 and the mannose receptor MRC1). M2 also produce abundant levels of the chemokines CCL17, CCL22 (Bonecchi et al. 1998) and CCL18 (Bonecchi et al. 2011) that in turn favor and sustain a Th2 response and tumor growth. Furthermore, M1 and M2 have distinct metabolic properties, as demonstrated by the dichotomic metabolic pathways of arginine (Munder et al. 1998; Hesse et al. 2001) and iron (Recalcati et al. 2010; Cairo et al. 2011). Major functions of M2 are their contribution in the clearance of parasites, wound healing and tissue remodeling, suppression of T-cell proliferation, pro-tumoral activity by virtue of their immunoregulatory and angiogenic abilities (Biswas & Mantovani 2010).

## 3. The tumor microenvironment: Macrophage polarity in Tumor-Associated Macrophages (TAM)

Several tumor-microenvironmental signals have been reported to instruct TAM polarization, including prostaglandin E2 (Rodriguez et al. 2005; Hagemann et al. 2006; Eruslanov et al. 2009; Eruslanov et al. 2010), migration-stimulating factor (MSF) (Solinas et al. 2010), and TGFβ (Flavell et al. 2010). Strong evidence also supports the relevance of M-CSF as a pro-tumoral factor attracting and triggering a M2-like polarization within the tumor. Indeed in human tumors, overproduction of M-CSF is associated with a poor clinical outcome in a wide range of cancers and several tumor types feature characteristics of an M-CSF-induced gene expression signature (Espinosa et al. 2009; Webster et al. 2010). This is consistent with transcriptional profiling analysis on *in vitro* differentiated macrophages, which have shown that M-CSF differentiated macrophages exhibit M2-like features, while GM-CSF differentiated macrophages exhibit M1-like features (Martinez et al. 2006; Fleetwood et al. 2007; Hamilton 2008). Accumulating experimental evidence from murine models of cancer further showed the pro-tumoral role of M-CSF. For example transplanted tumors' growth is impaired in M-CSF-deficient mice (Nowicki et al. 1996) and blockade of either M-CSF or is receptor leads to impaired tumor growth (Aharinejad et al. 2004; Kubota et al. 2009; Priceman et al. 2010). On the other hand, whereas no effect on tumor development was observed in the spontaneous mammary cancer model MMTV-PyMT in an M-CSF deficient background, the development of metastatic carcinoma was delayed (Lin et al. 2001). Angiogenesis is clearly a pro-tumoral feature as it provides the necessary "fuel" favoring tumor growth. In this context M-CSF has been shown to exert a pro-angiogenic effect in macrophages by inducing the production of VEGF (Curry et al. 2008).

A second pathway skewing TAM to the M2 phenotype is sustained by the Th2-derived cytokines IL-4 and IL-13. In the spontaneous mammary carcinoma model driven by PyMT both cytokines have been shown to be responsible for the M2 polarization of TAMs (DeNardo et al. 2009). In this model IL-4 derived from $CD4^+$ T cells and IL-13 derived from NKT cells instruct TAM an M2-like polarization leading to tumor development. Conversely, blockade of IL-4Rα led to a diminished M2-like gene expression profile and a switch to M1-associated gene expression, ultimately resulting in increased tumor surveillance. IL-4-induced M2 polarization of TAM has also been evidenced in a model of pancreatic cancer (Gocheva et al. 2010). IL-4 induced the activity of cathepsin in TAM, resulting in increased angiogenesis and tumor growth. The contribution of IL-13 to the M2 polarization of TAM has been demonstrated in the 4T1 mammary carcinoma model (Sinha et al. 2005). In this

context, macrophages from CD1d-deficient mice (that lack NKT cells) show a M1 tumoricidal phenotype and metastasis resistance. IL-10 is also well known to induce an M2 phenotype. In tumor, IL-10 produced by Treg cells has been shown to dampen TAM capacity to mount a T cell mediated immune response (Kuang et al. 2009). B cells are also a source of tumoral IL-10. It has been demonstrated that IL-10 production by B-1 cells induced a M2 polarization of TAMs in a B16 melanoma model (Wong, S. C. et al. 2010). Besides the contribution of B cells-derived IL-10 in driving M2 polarization of TAM, new evidence support that B cells skew TAM to a M2 phenotype via production of T cell-dependent autoantibodies against an extracellular matrix component in a K14-HPV16 skin carcinogenesis model (de Visser et al. 2005; Andreu et al. 2010).

Finally, emerging evidence indicate that besides their major role in monocyte recruitment chemokines, CCL2 (MCP-1) in particular, may also be involved in macrophage polarization in the tumor burden (Roca et al. 2009). Indeed, human $CD11b^+$ peripheral blood mononuclear cells induced to differentiate upon CCL2 stimulation upregulated M2 markers such as CD14 and CD206 (also known as Mannose Receptor 1). This M2 polarizing effect and thus pro-tumoral role of CCL2 is paralleled with the observation that many tumors overexpress CCL2 and these high levels have been associated with a bad outcome in cancer patients (Qian & Pollard 2010). It was furthermore recently reported in a murine breast-cancer model that CCL2 induced inflammatory monocytes infiltration in tumors (Qian et al. 2011). Moreover, tumor cells-derived CCL2 was shown to play a prominent role in metastasis development.

## 4. Cell lineage commitment: Monocyte subsets

Experimental evidence highlights that macrophage plasticity depends not only on the specific microenvironment encountered upon their extravasation from the circulation, but also on the existence of myelomonocytic subsets and lineage-committed TAM subpopulations that exploit diverse tumor-promoting activities (Coffelt et al. 2010b; Geissmann et al. 2010a; Geissmann et al. 2010b). On the basis of morphology and differential expression of antigenic markers, three types of blood monocytes (classical, intermediate, and nonclassical) have been described for both human and murine system (Ziegler-Heitbrock et al. 2010). In the mouse monocytes, which express CD115 (M-CSF receptor) and CD11b (Mac 1), are classified based on the expression level of Ly6C (one of the epitopes recognized by the anti-Gr-1 monoclonal antibody) in $Ly6C^{high}CD43^+$ or classical monocytes, $Ly6C^{high}CD43^{++}$ or intermediate monocytes and $Ly6C^{low}CD43^{++}$or nonclassical monocytes. These two subsets have been demonstrated to have different functions and migration patterns (Auffray et al. 2009), as classical monocytes are $CX3CR1^{low}CCR2^+CD62L^+$ and are actively recruited to sites of inflammation whereas nonclassical monocytes were $CX3CR1^{hi}CCR2^-CD62L^-$ and make homing to non-inflamed tissues. Recently, Geissman and colleagues demonstrated that the nonclassical monocyte subset constantly patrols the blood vessel wall and can be rapidly recruited to sites of inflammation before the arrival of classical monocytes (Auffray et al. 2007). The developmental relationship between the two monocyte subsets is still unclear. Experimental data suggest the possibility of a common precursor that gives rise to both classical and nonclassical monocytes. Adoptive transfer of classical monocytes demonstrated that this subset decreased the expression of Ly6C giving rise to nonclassical monocytes (Yrlid et al. 2006; Varol et al. 2007; Movahedi et al. 2010).

However, the generation of nonclassical monocytes has not been affected by antibody-mediated depletion or genetic defect in classical monocytes (Scatizzi et al. 2006; Feinberg et al. 2007; Mildner et al. 2007; Alder et al. 2008). Monocyte subsets were also identified in the human settings, with some consistency and some discrepancies as compared to the murine setting. Three human monocyte subsets were defined based on the expression levels of CD14 and CD16 (the FcγRIII molecule): classical monocytes ($CD14^{++}CD16^{-}$), intermediate monocytes ($CD14^{++}CD16^{+}$) and non-classical monocytes ($CD14^{+}CD16^{++}$). Gene expression profiles of these subsets indicates that they exhibit common gene expression patterns (at intermediate levels mirroring the CD14/CD16 levels) but also display unique features that potentially argue for distinct roles of these subsets in the immune process (Wong, K. L. et al. 2011; Zawada et al. 2011). Classical and non-classical monocytes have both pro-inflammatory activities for examples in response to LPS challenge but differ in the cytokine/chemokine repertoire they produce in response to LPS (Wong, K. L. et al. 2011). Moreover, non-classical monocytes show "patrolling" properties and appear to be very responsive to virus stimulation (Cros et al. 2010). So far, no specialized function has been assigned for intermediate monocytes but it is of note that their frequency is increased in cardiovascular diseases (Heine et al. 2008; Rogacev et al. 2011) and HIV (Ellery et al. 2007; Jaworowski et al. 2007). Tie2-expressing monocytes (TEM) were found in the nonclassical monocyte subset (De Palma et al. 2005). These monocytes play a non-redundant role in tumor neovascularization as their selective depletion resulted in reduced tumor angiogenesis in murine tumor models. TEM are selectively recruited to tumors by the Tie2 ligand angiopoietin-2 (ANG-2), which is expressed by tumor endothelium (Venneri et al. 2007; Coffelt et al. 2010a). Recent results indicate that Tie2 can be expressed also by classical and intermediate human monocytes (Zawada et al. 2011).

A discussed issue about monocyte heterogeneity is about identity and localization of their precursors. A compartmental reservoir of extramedullary monocytes has been identified in the subcapsular red pulp of the spleen (Swirski et al. 2009). These undifferentiated monocytes express Ly6C, rapidly amplified and are recruited to ischemic myocardium while their role in the tumor context is still unknown. Mobilization and proliferation of precursors in peripheral tissues has been studied as another mechanism to give rise to differentiated macrophages (Massberg et al. 2007). CCR2 ligands seem to play a central role for the mobilization of committed hematopoietic precursors to peripheral sites where they differentiate into M2 repair macrophages (Si et al. 2010). Hematopoietic precursors were found also in some tumor bearing-mice models (Kitamura et al. 2007; Deak et al. 2010) and probably they contribute to the mature macrophage pool. $CD34^{+}$ hematopoietic progenitors in presence of breast cancer cell culture medium differentiate in $CD11b^{+}$ myeloid cells that seem to be involved in the tumor angiogenesis and in the initiation of premetastatic niche. Proangiogenic $CD11b^{+}$ monocytes have been identified in the blood of tumor-bearing mice and cancer patients (Laurent et al. 2011).

At present our understanding of the relative role of monocyte subsets in tumor infiltration and biology is still largely incomplete. Classical monocytes represent one of the cellular components of a heterogeneous population of myeloid nature indicated as myeloid-derived suppressor cells (MDSC), which also includes immature monocytes and granulocytic cells (Sinha et al. 2007; Gabrilovich & Nagaraj 2009; Peranzoni et al. 2010). MDSC are functionally defined for their immunosuppressive functions, are expanded both at tumor site and in

secondary lymphoid organs in tumor-bearing animals and in cancer patient blood samples, where their increase correlated with the clinical cancer stage (Diaz-Montero et al. 2009). Recently it has been demonstrated that classical monocytes preferentially infiltrate lung tumor metastasis, while nonclassical monocytes are mainly recruited to primary tumor site (Qian et al. 2011).

## 5. An integrated view

The different circulating monocyte subsets identified appear to be committed to distinct extravascular fates in the tumor microenvironment (Figure 2). Classical monocytes are thought to differentiate mainly toward M2-like macrophages (Sinha et al. 2007; Geissmann et al. 2010b), and several studies have showed that MDSC in the tumors differentiate into immunosuppressive TAM (Kusmartsev & Gabrilovich 2005; Movahedi et al. 2010) and tolerogenic dendritic cells (Liu et al. 2009; Augier et al. 2010). Conversely, in TS/A and 4T1 tumors classical monocytes have been shown to include the precursors of both M1-like TAM enriched in hypoxic regions of the tumor and M2-like macrophages (Movahedi et al. 2010). As TEM are concerned, it is interesting to note that ANG-2 induces an M2-like phenotype (Pucci et al. 2009). However, TEM depletion has no impact on TAM recruitment in murine tumor models (De Palma et al. 2005), suggesting that TEM likely represent a sub-population of monocytes distinct from TAM precursors. Finally, despite monocytes have long been considered the unique precursors of macrophages, local proliferation of tissue-resident macrophages has been demonstrated for many populations, as alveolar macrophages (Sawyer et al. 1982; Tarling et al. 1987; Landsman et al. 2007), splenic white-pulp and metallophilic macrophages (Wijffels et al. 1994), and liver Kupffer cells (Crofton et al. 1978),

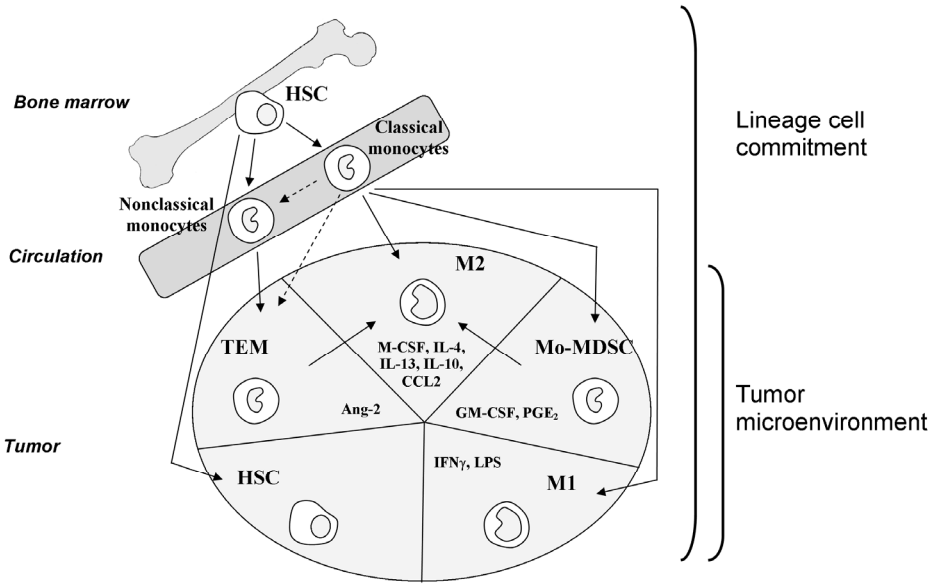



Fig. 2. Lineage commitment and tumor microenvironment in the generation of mononuclear phagocytes heterogeneity at the tumor site.

principally in homeostatic conditions. Of note, Allen and colleagues have also recently described IL-4-driven local proliferation of resident macrophages during helminth infections (Jenkins et al. 2011). In the context of cancer, it would thus be of great interest to address the potential effect of IL-4 on TAM proliferation to get a better insight in de novo recruitment of monocytes/macrophages versus local expansion of the existing pool of TAM.

The two main murine subsets, classical (Ly6C$^{high}$) and non-classical (Ly6C$^{low}$) monocytes, originate from hematopoietic precursors (HPC) in bone marrow and enter the tumor mass. Once in the tumor, exposure of monocytes and macrophages to different stimuli drive their polarization and function, resulting in the generation of the heterogeneous infiltrate. It remains unknown whether Ly6C$^{low}$ nonclassical monocytes are generated through a Ly6C$^{high}$ intermediate (dotted lines).

## 6. Conclusion

Macrophages are heterogeneous and plastic cells of the myelomonocytic lineage which adapt to the microenvironmental cues by changing their transcriptional program. Using cancer as a paradigm for macrophage polarization leads to the current view that various, if not all tumor-associated signals/factors/cytokines/chemokines/growth factors lead to a macrophage phenotype closely but at the same time different from the M2 type. Besides TAM, in tumors other monocyte/macrophages populations have been described, including MDSC, HPC and TEM, which display molecular and functional signatures resembling circulating monocytes subsets.

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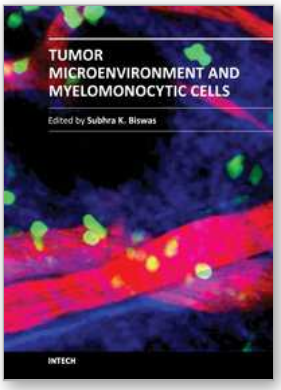


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Tumor microenvironment represents an extremely dynamic niche shaped by the interplay of different cell types (e.g. tumor cells, stromal cells), their soluble products (e.g.cytokines, chemokines and growth factors) and varied physico-chemical conditions (e.g low oxygen concentration or hypoxia). Recent studies have identified myelomonocytic cells as key players in regulating the tumor microenvironment and hence, tumor progression in a variety of cancers. In view of these findings, the present book attemps to provide a comprehensive account of the diversity of tumor microenvironment across different cancers and how myelomonocytic cells have taken the center-stage in regulating this niche to direct cancer progression. A better understanding of the myelomonocytic cells and the mechanisms by which they regulate cancer progression will open new vistas in cancer therapeutics.



**InTech Europe**
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821