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# TUMOR-ASSOCIATED MACROPHAGES AND DENDRITIC CELLS AS PROTOTYPIC TYPE II POLARIZED MYELOID POPULATIONS

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**Environmental signals polarize mononuclear phagocytes which can express different functional programmes. Fully polarized type I and type II (or alternatively activated) macrophages are the extremes of a continuum of functional states. Tumor-derived and T cell-derived cytokines stimulate tumor associated macrophages (TAM) to acquire a polarized type II phenotype. These functionally polarized cells, and similarly oriented or immature dendritic cells present in tumors, play a key role in subversion of adaptive immunity and in inflammatory circuits which promote tumor growth and progression.**

**Key words: dendritic cells inflammation, immunity, tumors macrophages.**

Cells belonging to the monocyte-macrophage lineage have long been recognized to be heterogeneous. For instance, macrophages obtained from certain anatomical sites exhibit peculiar morphology and function as exemplified by lung alveolar macrophages. Since lineage-defined subsets have not been identified to-date, macrophage heterogeneity is likely to reflect the plasticity and versatility of these cells in response to exposure to microenvironmental signals. Cytokines and microbial products profoundly affect the function of mononuclear phagocytes. In particular, cytokines associated with polarized type II responses (IL-4, IL-13, IL-10) induce an alternative activation program in macrophages. "Alternatively activated"[1,2,3] or type II macrophages show distinct functional properties which integrate them in polarized type II responses.

Macrophages are a major component of the leukocyte infiltrate of tumors[4,5]. Tumor-associated macrophages (TAM) originate from circulating blood monocytes. Their recruitment and survival *in situ* is directed by chemokines[4] and by cytokines which interact with tyrosine kinase receptors. TAM have complex dual functions in their interaction with neoplastic cells (the "macrophage balance" hypothesis)[4] but strong evidence suggests that they are part of inflammatory circuits that promote tumor progression[4,5].

Here we will concisely review the properties of polarized macrophages and summarize recent information consistent with the view that TAM are a polarized type II (or M2 or alternatively activated) macrophage population[1,2,6]. The characteristics of dendritic cells (DC) which are at least in part related to the myelo-monocytic differentiation pathway, infiltrating neoplastic tissues will also be considered. The polarized functions of TAM integrate them in circuits of subversion of antitumor immunity and in inflammatory mechanisms which promote tumor growth and progression.

## 1. Dichotomy of macrophage polarization

Macrophage activation in response to microbial agents and cytokines, interferon-γ (IFNγ) in particular, has long been recognized[7]. More recently, it was realized that antiinflammatory molecules, such as glucocorticoid hormones, IL-4, IL-13 and IL-10, are more than simple inhibitors of macrophage activation, in that they induce a distinct activation program (alternatively activated macrophages)[1,2,3]. In analogy with the Th1/Th2 dichotomy in T cell responses, macrophages exposed to IFNγ and IL-4 have also been referred to as M1 and M2[6]. Here we will refer to these polarized ends of the spectrum of macrophage activation as type I and type II cells, or M1 and M2.

Polarized macrophages differ in terms of receptor expression, cytokine and chemokine production, effector function. Macrophages exposed to the classic activation signals IFNγ and LPS express opsonic receptors (eg, FcgRIII/CD16) whereas M2 cells are characterized by abundant levels of non-opsonic receptors (eg the mannose receptor). Arginine metabolism is characterized by high levels of inducible NO synthase (iNOS) in type I macrophages, whereas the arginase pathway predominates in the type II polarized macrophages with generation of ornithine and polyamines.

Differential cytokine production is a key feature of polarized macrophages. The type I phenotype includes IL-12 and TNF while type II macrophages typically produce IL-10 and IL-1 receptor antagonist (IL-1ra). Components of the IL-1 system are differentially regulated in polarized macrophage populations. IL-4, IL-13 and glucocorticoid hormones induce expression of the

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IL-1 type II decoy receptor whereas IFNγ and LPS inhibit it. The latter signals upregulate the signaling type I receptor, and IL-1R accessory protein (IL-1RacP)[8]. IL-4 and IL-13 induce IL-1ra production and inhibit IL-1[9]. Therefore, pro and antiinflammatory components of the IL-1 system are coordinately regulated by signals that polarize macrophages in a type I or type II direction.

Components of the chemokine network are differentially modulated in polarized macrophages. IL-10 up-regulates the CC chemokine receptors CCR1, CCR2 and CCR5. In contrast, CXCR2 and CXCR4 are partially downregulated under the same conditions[10]. An increase of CCR2 expression is also observed in monocytes exposed to dexamethasone[11]. IL-4 and IL-13 do not modify the expression of CC chemokine receptors but induce functional CXCL8 (IL-8) receptors in human monocytes[12]. On the contrary, monocytes exposed to LPS or IFNγ down-regulated CCR1, CCR2 and CCR5[13,14]. Similarly to what reported for DC[15], exposure of monocytes to classical pro-inflammatory signals induces the expression of functional CCR7 and the effect is inhibited by IL-10 (Sozzani, unpublished). Cytokine mediators that polarize macrophages in a type II direction inhibit the production of various chemokines. Production of IL-8/CCL8, RANTES/CCL5 and MIPs as well as of the IFNγ-inducible chemokines IP-10/CXCL10 and MIG/CXCL9 are inhibited by IL-4 and IL-10[16]. On the contrary, IL-4 selectively induces eotaxin-2/CCL24[17], CCL18 and MDC/CCL22 in macrophages and DC and these effects are inhibited by IFNγ[18,19]. CCL18 is also up regulated by IL-10 and antiinflammatory drugs[19,20]. Therefore, differential production of chemokines which attract Th1 (CXCL9, CXCL10) and Th2 or T regulatory cells (CCL22) integrates type I and type II macrophages in circuits of amplification and regulation of polarized T cell responses.

Different inducers of type II polarization, elicit distinct functional phenotypes in macrophages. Given the fact that inducers like IL-4/IL-13, IL-10, glucocorticoid hormones, vitamin D3, use widely different receptors and signaling pathways, this is hardly surprising. Moreover, mononuclear phagocytes are exposed to a multiplicity of signals *in vivo* with different temporal patterns. Therefore, polarization of macrophage function should be viewed as an operationally useful, simplified conceptual framework describing a continuum of diverse functional states. With this general caveat, available information suggests that classically activated type I macrophages (M1) are potent effector cells which kill microorganisms and tumor cells and produce copious amounts of proinflammatory cytokines. In contrast, type II macrophages (M2) tune inflammatory responses and adaptive Th1 immunity, scavenge debris, promote angiogenesis, tissue remodelling and repair.

## 2. Tumor-associated macrophages (TAM)

### *2.1 Origin*

TAM derive from circulating monocytic precursors and *in situ* proliferation is generally not an important mechanism that sustains the mononuclear phagocyte population, at least in human tumors[4]. Several lines of evidence, including correlation between production and infiltration in murine and human tumors, passive immunization and gene modification, indicate that chemokines play a pivotal role in the recruitment of monocytes in neoplastic tissues[21]. Indeed, tumors have been invaluable for the discovery of several members of the chemokine superfamily. Chemokines are usually classified according to their constitutive (eg, CXCL12) or inducible production (eg, CCL2, CXCL8). Tumors are generally characterized by the constitutive expression of chemokines belonging to the inducible realm[22]. The molecular mechanism accounting for constitutive expression have been defined only for CXCL1 and involved NF-κB activation. Melanoma cells display high expression of NF-κB-inducing kinase (NIK)[23] and this phenotype is responsible for constitutive activation of IkB kinase activity and MAPK signaling cascades, as well as for constitutive activation of NF-κB[24]. This may represent a general mechanism underlying constitutive expression of inflammatory chemokines in tumors.

CCL2 is probably the most frequently found CC chemokine in tumors, since its description as a tumor-derived chemotactic factor[25]. Human tumors shown to express CCL2 *in vivo* include sarcomas, gliomas, lung tumors, carcinomas of the breast, cervix and ovary, melanomas. A recent careful analysis of the impact of CCL2 on tumor growth in a non tumorigenic melanoma system revealed a biphasic effect[26]. Low-level CCL2 secretion, with "physiological" accumulation of TAM, promoted tumor formation, while high CCL2 secretion resulted in massive macrophage infiltration into the tumor mass and in its destruction[26]. These results in a model of melanoma progression are consistent with the "macrophage balance" hypothesis[4] and emphasize the protumor potential of levels of macrophage infiltration similar to those observed in human malignant lesions.

CCL5 is produced by breast carcinoma and melanoma[27,28]. In breast carcinoma CCL5 expression by tumor cells correlates with a more advanced stage of disease, suggesting that CCL5 may be involved in breast cancer progression[28,29]. A variety of other chemokines have been detected in neoplastic tissues as products of tumor cells or stromal elements. These include CXCL12, CXCL8, CXCL1, CXCL13, CCL17 and CCL22. CXCL1 and related molecules (CXCL2, CXCL3, CXCL8 or IL-8) have been shown to play an important role in melanoma progression[30]. They do so by direct stimulation of neoplastic growth, promotion of inflammation and induction of angiogenesis. CXCL8 and related chemokines act primarily on neutrophils. However, IL-4 and IL-13 render monocytes exquisitely sensitive to CXCL8 and CXCL1[12]. Therefore, in the tumor microenvironment where polarized type II T cells can be present, CXCL8 and related chemokines may contribute to guide the positioning and to regulate the function of TAM. Inspite of constitutive production of CXCL8 and related chemokines by tumor cells, neu-

trophils are not a major and obvious constituent of the leukocyte infiltrate. However, these cells, though present in minute numbers, may play a key role in triggering and sustaining the inflammatory cascade.

Vascular endothelial growth factor (VEGF) and macrophage colony stimulating factor (M-CSF) are cytokines commonly produced by tumors, which interact with tyrosine kinase receptors and elicit monocyte migration. There is evidence that M-CSF and VEGF can significantly contribute to macrophage recruitment in tumors[31-33]. These molecules also promote macrophage survival and proliferation, the latter generally limited to murine TAM. Studies in M-CSF-deficient mice (op/op) have provided strong support to the concept of the protumor function of the mononuclear phagocyte system. It was originally reported that M-CSF-deficiency in op/op mice diminishes macrophage recruitment, stroma formation and tumor growth in the Lewis lung carcinoma model[34]. In a mammary carcinoma model, M-CSF- deficiency did not affect early stages of tumor development, but reduced progression to invasive carcinoma and metastasis[31]. Genetic restoration of M-CSF production in epithelial cells restored macrophage infiltration as well as malignant behavior.

Chemokines in tumors are more than leukocyte attractants. Transcriptional profiling has shown that CC chemokines activate a restricted and distinct program in human monocytes[35]. The chemokine activated transcriptional profile includes matrix metalloproteases (see below for discussion) and cytochrome CYP1B1, involved in carcinogenesis. Moreover, CXC chemokines with an ELR motif stimulate angiogenesis[36]. Tumor cells express receptors for chemokines[28,37-39] and can respond to these mediators with increased proliferation and survival. Finally, chemokine-driven leukocyte recruitment results in digestion of the extracellular matrix which paves the way for tumor cell leaving the primary lesions (counter-current invasion)[40]. Chemokine receptors can then guide localization of neoplastic elements at distant anatomical sites[37-39]. Interestingly, recent evidences have shown that plasminogen production by sarcoma cancer cells acts as a negative regulator of TAMs recruitment[41]. Therefore, TAM recruitment is likely to be the net effect of chemotactic and repulsive inhibitory signals within the neoplastic tissues.

### *2.2 Survival and differentiation*

It is likely that sustaining macrophage survival in tumors contributes to the levels of infiltration. CSFs, and M-CSF in particular, are likely to promote the macrophage lifespan as well as, in some murine tumors, proliferation of TAM[4,31-33]. Recently, placenta-derived growth factor (PlGF), a molecule related to VEGF in terms of structure and receptor usage, has been reported to promote the survival of TAM[42].

TAM isolated from various murine tumors and from human ovarian cancer express very low level of inflammatory chemokine receptors, CCR2 in particular[43]. It has been shown that primary inflammatory signals, including TNFα, downregulate CCR2 in monocytes[13] (Figure 1). Therefore, downregulation of CCR2 may in part reflect the monocyte-to-macrophage transition and, in part, TNFα present in the ovarian cancer microenvironment[44]. Downregulation of CCR2 may serve as a mechanism to localize and retain macrophages in tumors.

Cytokines present in the tumor microenvironment have the potential to promote and orient the differentiation of recruited mononuclear phagocytes. IL-10 as well as TGFβ are produced by a variety of tumor cells (including ovarian cancer) and by TAM themselves[45-49]. IL-10 has been shown to promote the differentiation of monocytes to mature macrophages and to block their differentiation to DC[50]. The effect of IL-10 on monocyte differentiation may be an important determinant of the relative proportion of TAM *versus* tumor-associated DC (TADC) and of their relative distribution. For instance, in papillary carcinoma of the thyroid, TAM are evenly distributed throughout the tissue, in contrast to DC which are present in the periphery[51]. A gradient of tumor-derived IL-10 may account for differentiation along the DC *versus* the macrophage pathway in different microanatomical localizations in a tumor. To the extent that they have been investigated, differentiated mature TAM have phenotype and function similar to type II macrophages. TAM from poorly immunogenic malignant tumors have little cytotoxicity for tumor cells and they actually promote tumor cell proliferation in particular under suboptimal culture conditions[4]. In apparent contrast, a recent report has shown that TAM from various cancers appear to be activated by cancer cells to produce the TNF-related apoptosis-inducing ligand (TRAIL) and to induce the expression of the TRAIL death receptors DR4 and DR5 on tumor cells[52]. However, in this study, pleural effusion cells were studied rather then cells from solid neoplasms. Further work is needed to investigate expression and significance of TRAIL in TAM.

TAM are poor producers of NO[53] and, *in situ* in ovarian cancer, only a minority of tumors and, in these, a mi-

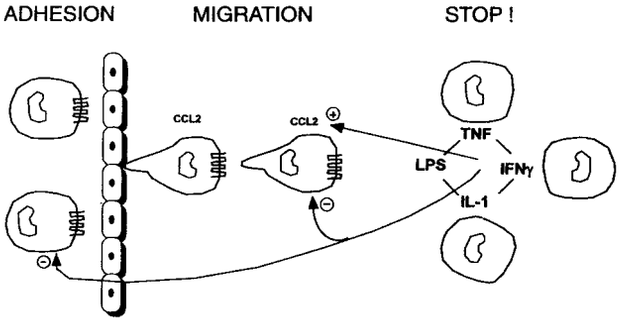


Figure 1 - A stop signal in the recruitment of TAM. Inflammatory signals (eg, TNF in ovarian carcinoma) present in the tumor microenvironment downregulate expression of CCR2 in recruited monocytes. When these molecules leak into the systemic circulation as in advanced cancer, they can paradoxycally inhibit inflammation at distant sites.

nority of macrophages localized at the periphery scored positive for iNOS[54]. Moreover, in contrast to M1 polarized macrophages, TAMs have been shown to be poor producers of reactive oxygen intermediates (ROIs), compared to normal macrophages, consistent with the hypothesis that these cells represent a skewed M2 population[55].

Arginase expression in TAM has not been studied. However, it has been recently proposed that the carbohydrate-binding protein galectin-1, which is abundantly expressed by ovarian cancer[56] and shows specific anti-inflammatory effects, promote the classic pathway of L-arginine resulting in a strong inhibition of the nitric oxide production by lipopolysaccharide-activated macrophages[57]

TAM have high mannose receptor (Allavena P, unpublished) and are poor at presenting antigen[4]. As discussed below, they have an IL-10 $^{high}$ IL-12 $^{low}$ cytokine repertoire.

Uneven vascularization and hypoxia are characteristics of neoplastic tissues which affect macrophage distribution and function. TAM accumulate preferentially in the poorly vascularized region of tumors which are characterized by low oxygen tension. Recent studies have shown that macrophage migration is suppressed in hypoxic condition[58,59] and that TAM are immobilized in avascular[60] and necrotic hypoxic areas of tumors[61]. Evidence suggests that in hypoxia TAM are stimulated to co-operate with tumor cells and promote angiogenesis[62]. Thus, hypoxia represents a stress factor that, along with other microenvironmental parameters, such as low pH, low glucose levels and high lactate levels, deeply affects the biology of TAM. Expression of the Hypoxia-inducible factor-2α (HIF-2α) was observed in TAM from breast carcinoma[63], and HIF-1 is produced by macrophages in hypoxia *in vitro*, and in avascular areas of breast carcinoma[64]. Under low oxygen conditions, TAM switch their metabolism to anaerobic pathways and this adaptation is achieved by the increased expression of hypoxia inducible genes, such as VEGF, bFGF and CXCL8, whose transcription is controlled by the transcription factor HIF-1 and which stimulate angiogenesis. The relevance that the hypoxia-dependent pathways play in macrophage functions was recently demonstrated by Cramer *et al.*[65] By using conditional knockouts of the hypoxia responsive transcription factor HIF-1α, these authors have provided *in vivo* demonstrations that in macrophages this factor is essential to generate ATP in low-oxygen conditions and to promote functions such as migration and antibacterial activity. This observation highlights the relevance that the hypoxia-HIF-1α pathway may play in the recruitment and activation of TAM into solid tumors.

T cells infiltrating various types of human tumors have a type II phenotype, with a predominance of CD8+ (eg, Kaposi's sarcoma) or CD4+ cells (eg, cervical carcinoma) in different neoplasms[5]. By producing IL-4, IL-13 and IL-10, tumor infiltrating T cells may reinforce the skewing of monocyte differentiation in tumors towards a type II phenotype.

### *2.3. In the light of adaptive immunity: the chemokines*

As mentioned above, chemokines are more than leukocyte attractants. Chemokine are part of amplification and regulation systems of polarized T cell responses. Work in gene-modified mice has shown that CCL2, produced by a variety of tumors, can orient specific immunity in a Th2 direction, although the exact mechanism for this action has not been defined[66]. It may include stimulation of IL-10 production in macrophages. In addition to being produced by neoplastic cells, CCL2 is also expressed at high levels in TAM (Sica A, unpublished results). Antibody blocking experiments have provided direct evidence that CCL2 secretion in a poorly immunogenic tumor blocks the generation of tumor-reactive T cells[2]. Reed-Sternberg cells in Hodgkin's lymphoma have been shown to express CCL22 (MDC) and CCL17 (TARC)[67,68]. These chemokines recognize CCR4 which is preferentially expressed on polarized Th2 cells and on T regulatory cells (Treg) as well as on monocytes[22,69]. Interestingly, in the same tumor, stromal cells produce CCL11 (eotaxin), which attracts eosinophils and Th2 cells. Therefore, in this human tumor neoplastic elements and stroma use complementary tools to recruit cells associated with polarized type II responses. In the same vein of driving into tumors polarized Th2 cells, the oncogenic virus human herpesvirus 8 (HHV8), involved in the pathogenesis of Kaposi's sarcoma and hematological malignancies, encodes three CC chemokines (vMIPI, II and III) which interact as agonists with CCR3, CCR4 and CCR8 and, accordingly, preferentially attract polarized type II T cells[5] and, presumably, Treg cells[69]. Consistently with these *in vitro* observations, Kaposi's sarcoma is infiltrated by CD8+ and, to a lesser extent, CD4+ cells with a predominant type II phenotype. Therefore HHV8 virus-encoded chemokines represent a strategy to subvert effective antiviral/antitumor immunity by favouring type 2 responses and, possibly, Treg cells.

In addition to being a target for chemokines, TAM are a source of a selected set of these mediators (CCL2, CCL22, CCL18). CCL18 was recently identified as the most abundant chemokine in human ovarian ascites fluid[70]. When the source of CCL18 was investigated, it was tracked to TAM, with no production by ovarian carcinoma cells. CCL18 is a CC chemokine produced constitutively by immature DC and inducible in macrophages by IL-4, IL-13 and IL-10[20]. Since IL-4 and IL-13 are not expressed in substantial amounts in ovarian cancer, it is likely that IL-10, produced by tumor cells and macrophages themselves, accounts for CCL18 production by TAM. CCL18 is an attractant for naive T cells by interacting with an unidentified receptor[2]. Attraction of naive T cells in a peripheral microenvironment dominated by type II macrophages and immature DC may induce anergy.

### *2.4. IL-10 and TGFβ and the tuning of adaptive immunity*

It has long been known that TAM have poor antigen presenting capacity and actually can suppress T cell ac-

tivation and proliferation[5]. The suppressive mediators produced by TAM include prostaglandins, IL-10 and TGFβ[45-49]. Moreover, they do not produce IL-12 spontaneously and they are refractory to stimulation by IFNγ and LPS. The IL-10 $^{high}$ IL-12 $^{low}$ phenotype is characteristic of polarized type II macrophages (see above). Antibody blocking experiments suggest that autocrine IL-10 in part accounts for the defective IL-12 production[45].

The mechanisms underlying the IL-10$^{high}$ IL-12$^{low}$ phenotype are complex. IL-10 derived from tumor cells and T cells may favour differentiation along this pathway, as discussed above. In a recent elegant study, Ibe *et al.*[71] have recently suggested that during tumor establishment T cells condition TAM to produce IL-10 and that inactivation of T cells results in a switch of TAM towards IFNγ production and elicits tumor rejection.

The molecular basis responsible for the unresponsiveness of TAM to stimulation of IL-12 production has been investigated[45]. TAM display a massive and costitutive nuclear localization of the NF-κB inhibitory p50 homodimer (Sica A, unpublished), which most likely provides a molecular mechanism for other alterations of TAM functions dependent on NF-κB activation, including defective iNOS and defective NO production expression[53,54].

Immature myeloid cells generated as a consequence of CSF production during tumor growth have been shown to be potent suppressors of T cell responses[72]. These immature myeloid suppressors have been shown to respond to undefined tumor attractants and to be present in the neoplastic tissue in head and neck squamous cell carcinoma[73]. The relation, if any, of immature myeloid suppressor cells with TAM remains to be defined.

### *2.5. Links to adaptive immunity: tumor-associated DC*

DC are at least in part related to the myelomonocytic differentiation pathway and some of them can originate from monocytes. Several reports have documented the presence of TADC in tumors[4,50,74]. TADC are differentially localized in tumors. In breast cancer it was found that immature langerin+ DC are interspersed in the tumor mass, whereas more mature CD83+, DC-LAMP+ DC are confined to the peritumoral area[75]. In papillary thyroid carcinoma TADC were localized at the invasion front of the tumor[51]. Interestingly, this distribution was clearly different from that of TAM which were evenly scattered in the tissue[51]. More recently ovarian tumors were reported to be infiltrated by a subset of DC, namely plasmacytoid DC[76].

Tumor cells express many CC chemokines that are likely involved in the recruitment of leukocyte to the tumor mass, including DC[5,74,77]. The CC chemokine MIP3α/CCL20 is a potent attractant of Langerhans-type DC, but not of monocyte-derived DC which do not express the cognate receptor CCR6. CCL20 has been shown to be expressed in pancreatic carcinoma, renal cancer, breast carcinoma and papillary thyroid carcinoma[51,75,78,79]. CCL20 was expressed by carcinoma cells as well as, in pancreatic cancer, by TAM[51,75,78]. Immature DC express receptors (eg, CCR1 and CCR5) for inflammatory chemokines and CCL5 has been suggested to be a major attractant for immature DC produced by papillary thyroid carcinoma[51]. In this tumor, hepatocyte growth factor amplifies chemokine production by interacting with the Met receptor[51]. Expression of CXCL12/SDF-1, the main chemotactic signal for circulating plasmacytoid DC[80] was reported to be associated to the recruitment of this DC subset to ovarian carcinoma lesions[76].

The actual significance of TADC for human tumor progression is uncertain. TADC generally show an immature phenotype with high CD1a and low costimulatory molecules CD80, CD86 and CD40[51,75,81-84]. Accordingly, in the few studies conducted so far it was found that TADC have defective allostimulatory activity[81-83]. When present, mature DC accumulate in peritumoral tissues, even after chemokine gene transfer[85]. Immature DC would induce tolerance rather than immune reactivity[86], and induce the generation of Treg cells that will inhibit the generation of cytotoxic T lymphocytes[87]. Indeed, *in vivo* depletion of CD4+ CD25+ Treg cells leads to the development of tumor-specific effector cells in mice that where otherwise unresponsive to tumors[88]. This immature phenotype of TADC may reflect lack of effective maturation signals *in situ*, prompt migration to lymph nodes of mature cells or the presence of maturation inhibitors in the tumor context. These elements may be operative to different degrees in different tumors. For instance, in papillary thyroid cancer there is evidence for substantial accumulation of Langerhans-type DC in regional lymph nodes, presumably reflecting migration from the tumor[51,89-92]. Many tumors produce IL-10, IL-6, TGFβ and M-CSF. IL-10 has been shown to block the differentiation and maturation of DC, including TADC[50]. Inhibition of DC differentiation from bone marrow precursors has also been observed with IL-6 and M-CSF[93]. One could speculate that immature DC may maintain tolerance to tumor antigens and that TADC, in analogy with TAM[4], may in some tumors promote tumor progression and dissemination. Production of PGE2 and release of ATP by dying cells in the tumor micro-environment might also contribute to the inhibition of DC maturation and IL-12 production[94-96].

### *2.6. Tissue remodeling, fibrosis and angiogenesis*

Phagocytes play a central role in tissue remodeling and repair during ontogenesis and adult life. This ancestral function of mononuclear phagocytes is expressed by TAM which orchestrate the function of other components of the tumor stroma. TAM produce a host of growth factors which affect tumor cell proliferation, angiogenesis, and the deposition and dissolution of connective tissues. These include epidermal growth factor (EGF), members of the FGF family, TGFβ, VEGF, chemokines. In lung cancer, TAM may favor tumor progression by contributing to stroma formation and angio-

genesis through their release of PDGF, in conjunction with TGF-β1 production by cancer cells[97].

Macrophages can produce enzymes and inhibitors which regulate the digestion of the extracellular matrix, such as MMPs, plasmin, urokinase-type plasminogen activator (uPA) and the uPA receptor. Direct evidence have been presented that MMP9 derived from hematopoietic cells of host origin contributes to skin carcinogenesis[98,99]. Chemokines have been shown to induce gene expression of various MMPs and, in particular, MMP9 production, along with the uPA receptor[35]. Induction of these molecules is part of a program of activation which arms monocytes with tools (receptors and enzymes) important for migration in tissues. Therefore, expression of enzymes and receptors which degrade the extracellular matrix could at least in part be induced and sustained by chemokines. Evidence suggests that MMP9 has complex effects beyond matrix degradation including promotion of the angiogenesis switch and release of growth factors[98,99]. Of relevance, distant primary tumors may induce premetastatic lung endothelial cells and macrophages to express MMP-9 via VEGFR-1/Flt-1 tyrosine kinase (TK), a mechanism which potentiates specifically pulmonary metastasis formation[100].

Angiogenesis is a key event in tumor growth and progression. Macrophages can exert a dual influence on blood vessel formation and function. On the one hand macrophages produce molecules that are proangiogenic, on the other hand they can express anti-angiogenic molecules and damage the integrity of blood vessels. On the anti-angiogenic side, in a murine model, CSF-induced, TAM-derived metalloelastase generates angiostatin[72]. In general, as for interaction with neoplastic cells, the pro-angiogenic functions of TAM prevail. In several studies, in human cancer TAM accumulation has been associated with angiogenesis and with the production of angiogenic factors such as VEGF and platelet-derived endothelial cell growth factor[5].

More recently, in human cervical cancer, VEGF-C production by TAMs was proposed to play a role in peritumoral lymphoangiogenesis and subsequent dissemination of cancer cells with formation of lymphatic metastasis[101]. Additionally, TAM participate to the proangiogenic process by producing the angiogenic factor thymidine phosporylase (TP), which promotes endothelial cell migration *in vitro* and whose levels of expression are associated with tumor neovascularization[102]. Moreover, TAM accumulate in hypoxic regions of tumors and hypoxia triggers a pro-angiogenic program in these cells (see above). Therefore, macrophages recruited *in situ* represent an indirect pathway of amplification of angiogenesis, in concert with angiogenic molecules directly produced by tumor cells.

Formation of a fibrous capsule and parasite encapsulation are late events associated with polarized Th2 responses. Fibrosis is a prominent feature of certain human tumors (eg, scirrhous carcinomas). CCL2 and IL-13, present in the tumor microenvironment as products of tumor cells, TAM or T cells, induce TGFβ production and fibrosis[103,104]. Therefore, it is tempting to speculate that polarized type II macrophages are part of the circuits that regulate the function of fibroblasts in the tumor stroma.

## 3. Overview

Mononuclear phagocytes are versatile, plastic cells that respond to environmental influences with the expression of distinct transcriptional programmes and functions. Transcriptional profiling is adding a new dimension to the definition of macrophage differentiation and heterogeneity (eg, ref 35 and unpublished data). Considerable progress has been made over the last few years in the characterization of TAM in diverse neoplastic tissues. The available information suggests that macrophages which infiltrate tumors acquire the properties of a polarized type II mononuclear phagocyte population.

The view of TAM as a skewed type II macrophage population is an oversimplification. Indeed tumors are a diverse set of disorders, and a systematic effort of *in vitro* and *in vivo* characterization has been made only in selected systems (eg, ovarian and breast cancer for human tumors). Moreover, available information indicates that the distribution and function of TAM and TADC differ considerably in different microregions of the neoplastic tissue (peripheral *versus* central; hypoxic *versus* normoxic areas). These limitations, as well as technological developments, call for gene expression profiling of infiltrating cells directly obtained from different regions of different tumors. Moreover, little dynamic information, analyzing function in relation to stages of progression, is available. In spite of these limitations, the view of TAM as a polarized M2 macrophage is efficacious in summarizing current understanding of the immunobiology of these cells.

By expressing properties of polarized type II macrophages, TAM participate in circuits that regulate tumor growth and progression, adaptive immunity, stroma formation and angiogenesis. In particular, TAM are a key component of inflammatory circuits which promote tumor progression and metastasis (for review, ref 5,105). The general hypothesis of a protumor role of inflammation, and of macrophages in particular, is supported by several lines of evidence including genetic analysis and gene targeting (discussed here and in ref 5,105). The available information suggesting that inflammatory reactions, and polarized infiltrating macrophages in particular, promote tumor progression, raises the possibility that the molecules and cells involved may represent novel, valuable therapeutic targets.

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