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ROLE OF TUMOUR-ASSOCIATED MACROPHAGES IN CANCER-RELATED INFLAMMATION

A. Sica[1,2,*]

[1]*Istituto Clinico Humanitas IRCCS, via Manzoni 56, 20089 Rozzano, Italy*

[2]*DiSCAFF, University of Piemonte Orientale A. Avogadro, via Bovio 6, 28100 Novara, Italy*

The construction of an inflammatory microenvironment provides the fuel for cancer development and progression. Hence, solid tumors promote infiltration of leukocyte populations, among which tumor-associated macrophages (TAM) represent a paradigm for cancer promoting inflammation. TAM orchestrate various aspects of cancer, including diversion and skewing of adaptive responses, cell growth, angiogenesis, matrix deposition and remodeling, the construction of a metastatic niche and actual metastasis, response to hormones and chemotherapeutic agents. Several evidence indicate that TAM show a remarkable degree of plasticity and functional heterogeneity, suggesting that during tumor progression macrophages undergo a phenotypic 'switch', eventually exhibiting the alternatively activated, 'M2', phenotype, associated with immunosuppression, promotion of tumor angiogenesis and metastasis. While recent studies have attempted to address the role of microenvironment signals on the TAM «reprogramming», the interplay between innate and adaptive immunity is emerging as a crucial step of this event. Here I discuss the evidence for the functional reprogramming of TAM during the course of tumor progression and the molecular mechanisms that regulate such event. Finally, I discuss the implications of this phenomenon for anti-cancer therapies aimed at prompting TAM to mount an effective antitumor response.

***Key Words*: tumor-associated macrophages, cancer-related inflammation.**

After a long eclipse, in recent years different lines of work have lead to a renaissance of the inflammation-cancer connection [1]. Selected forms of chronic inflammation predispose to cancer, including microbial infections (e.g. *Helicobacter pilori* for gastric cancer and mucosal lymphoma), autoimmune diseases (e.g. colitis-associated cancer), and inflammatory conditions of uncertain origin (e.g. prostate cancer). Usage of non-steroidal anti-inflammatory agents protects against various tumors. Moreover, smoldering inflammation is a key characteristic of the microenvironment of most neoplastic tissues, including those not etiologically related to inflammatory processes. Inflammation has been suggested to represent the 7th hallmark of cancer [2].

Recent efforts have shed new light on molecular and cellular pathways linking inflammation and cancer [1]. Two pathways link inflammation and cancer. In the intrinsic pathway, activation of different classes of oncogenes drives the expression of inflammation-related programs, which guide the construction of an inflammatory microenvironment. In the extrinsic pathway, inflammatory conditions promote cancer development (e.g. colitis-associated cancer of the intestine). Key orchestrators at the intersection of the intrinsic and extrinsic pathway include transcription factors (e.g. NF-κB, Stat3, hypoxia-inducible factor 1 (HIF)) [3], cytokines (e.g. TNF) and chemokines (Fig. 1). Thus, inflammation is a key component of the tumor microenvironment and a target for pharmacologic intervention.

Tumor-associated macrophages (TAM) are a major component of leukocytic infiltrate of tumors and have served as a paradigm for cancer-related inflammation. Macrophages are a double edged sword, with the potential to express pro and anti-tumor activity (the macrophage balance) [4], the former prevailing in established neoplasia. Macrophages and some of their products (IL-1; TNF; IL-6) have long been known to increase metastasis [1], and recent work has provided new evidence [5]. In particular, cells of hematopoietic origin and specifically myelomonocytic cells have been shown to home and condition the premetastatic niche. Here they form a secondary niche which favors secondary localization of cancer [1, 6, 7].

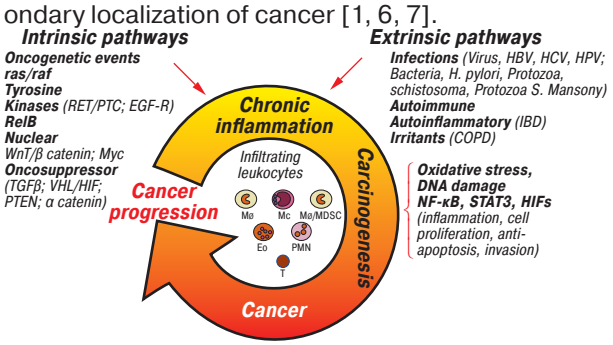

Fig. 1. Inflammation and cancer connection. Irrespective of the triggering event, both intrinsic (driven by genetic alteration) and extrinsic (driven by inflammatory cells and mediators) pathways result in inflammation and neoplasia. Both neoplastic cells and leukocytes, mainly belonging to the myelomonocytes lineage, contribute to the «smouldering» inflammation associated with tumor initiation and progression. The transcription factors NF-κB, HIF-1α and STAT-3 are key modulators of the inflammatory response that promotes cancer development through different mechanisms including induction of genomic instability, alteration in epigenetic events and subsequent inappropriate gene expression, enhanced proliferation and resistance to apoptosis of initiated cells, induction of tumor angiogenesis and tissue remodeling with consequent promotion of tumor cells invasion and metastasis. (Mø, macrophages; Mc, mast cells; MDSC, myeloid derived suppressor cells; Eo, eosinophils; PMN, polymorphonuclear cells; T, T lymphocytes).

Here I will focus on selected molecular pathways underlying TAM recruitment and polarization, empha-

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*Correspondence: Fax: +39 02 8224 5101;
E-mail: antonio.sica@pharm.unipmn.it

Abbrviations used: COX-2 – cyclooxygenase-2; IFNγ – interferon γ; HIF-1 – hypoxia-inducible factor 1; MDSC – myeloid-derived suppressor cells; TAM – tumor-associated macrophages.

sizing the dual potential of cancer-related inflammation [8] and the diversity of pathways and functions in different tumors.

INFLUENCE OF EXTRACELLULAR SIGNALS ON TAM FUNCTION AND POLARIZATION

Plasticity is a hallmark of mononuclear phagocytes. In response to diverse signals, macrophages undergo polarized activation. Classically activated macrophages (M1), following exposure to interferon γ (IFNγ), have antitumor activity and elicit tissue destructive reactions. In response to IL-4 or IL-13, macrophages undergo alternative activation (M2) [4]. In general, M2 cells obtained in response to IL-4 are oriented to tissue repair and remodeling, immunoregulation, and tumor promotion [4]. Interestingly, recent evidence indicates that polymorphonuclear leukocytes also can polarize in a type I or type II direction in cancer [9]. In hematopoietic tumors (e.g. multiple myeloma) macrophages promote survival and protect against cytotoxic chemotherapy [8]. Leukemic cells as well as hematopoietic stem cells share upregulated expression of CD47, a strategy to avoid recognition and phagocytosis by myelomonocytic cells [8].

In most, but not all investigated tumors, TAM have an M2-like phenotype [8]. Recent reports have established that the acquisition of pro-tumoral M2 functions by TAM is driven by various cytokines and signals expressed within the tumor microenvironment [10]. Among these, IL-10, PGE2, TGF-β and CSF-1 were reported to induce the M2 polarization of macrophages. Based on the M1 versus M2 paradigm of macrophage polarization [4], inhibition of M2- and activation of M1-inducing signals (eg. IL-10 and CpG, respectively) was proposed as a possible strategy to restore the anti-tumor functions of TAM [11]. Recent investigations suggest that during tumor growth both innate and adaptive immune cells participate in guiding the pro-tumoral phenotype of TAM [12].

In a model of mammary carcinoma, myeloid-derived suppressor cells (MDSC) were shown to contribute to tumor progression by suppressing T-cell activation and inducing an M2-like phenotype of TAM, (increased IL-10 and decreased IL-12 production) [13].

The role of B cells in solid tumor development is well characterized, for example B cell-deficient mice (uMT) exhibited resistance to several type of syngeneic tumors, including EL4 lymphoma, MC38 colon carcinoma, and B16 or D5 melanoma [12]. A first original report on B cells and cancer progression, in a transgenic mouse model of inflammation-associated cancer (HPV16 mice) demonstrated their role in *de novo* epithelial carcinogenesis [14]. Adoptive transfer of B lymphocytes or serum into B cell-deficient/HPV16 mice restored innate immune cell infiltration into premalignant tissue and reinstated necessary parameters for full malignancy (e.g., chronic inflammation, angiogenic vasculature, hyperproliferative epidermis) [14]. These findings suggest a model in which B lymphocytes are crucial in establishing chronic inflammation associated with *de novo* carcinogenesis. However, as B cells do not infiltrate the precancerous tissues, it was proposed that infiltration and functions of innate immune cells must be orchestrated remotely [15], suggesting that lymphocyte-derived cytokines and/or antibodies may drive the cancer-promoting inflammation. Recently, utilizing the MMTV-PyMT model of mammary carcinogenesis, DeNardo *et al*. [16] demonstrated that $CD4^+$ T lymphocytes expressing the M2 polarizing cytokines IL-4 and IL-13 potentiate mammary adenocarcinoma metastasis by modulating the pro-tumor properties of TAM. In turn, TAM enhances the invasive potential of malignant mammary epithelial cells. Using the K14-HPV16 mouse model of squamous carcinogenesis, the same group has recently shown that B cells and humoral immunity foster cancer development by activating Fcγ receptors (FcγR) on resident and recruited myeloid cells [17]. A recent report suggests that B1 cells, but not B2 cells, polarize peritoneal macrophages to an M2 phenotype, characterized by impaired expression of LPS-induced pro-inflammatory genes (eg. *Tnfa, Ccl3, Il1b*), with upregulation of the anti-inflammatory gene *Il-10* [18]. Overall, this information contributes to identification of M2 phenotype of TAM as a point of convergence of various pro-tumoral pathways, and reinforces the idea of IL-10 as a major *in vivo* promoter of the M2 macrophage's pro-tumor functions (Fig. 2).

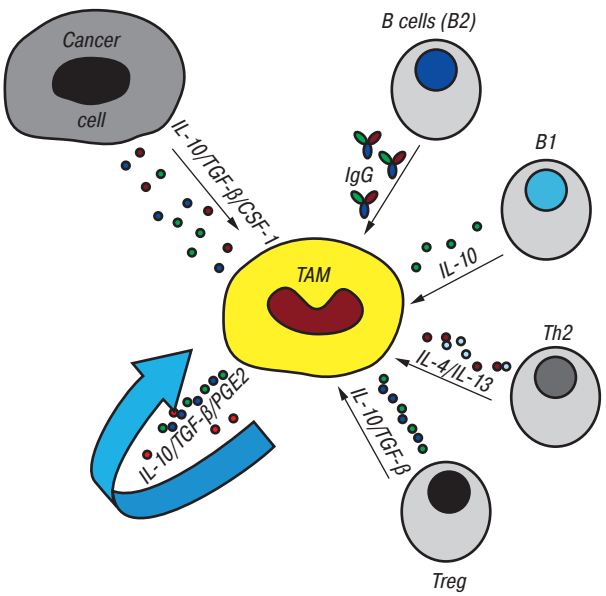

Fig. 2. Convergent pathways of M2 polarization of TAM. As depicted, along with tumor cells different leukocyte populations, including IL-10 producing B1 lymphocytes, concur to release cytokines and/or antibodies to promote the alternative M2 polarization of TAM. TAM itself contributes to creation an M2 polarizing microenvironment by autocrine production of M2 inducing signals. IL-10: interleukin-10; IL-4: interleukin-4; IL-13: interleukin-13; IgG: immunoglobulin G; CSF-1: colony stimulating factor-1; TGFβ: transforming growth factor-β; PGE2: prostaglandin E2 [1, 18, 28].

Indeed, in preclinical cancer models it was demonstrated that inhibition of IL-10 activity in combination with TLR agonists (eg. CpG) switched infiltrating macrophages from M2 to M1 and triggered innate immune response, debulking large tumors [3]. This emerging paradigm suggests that inhibition of mechanisms and

molecules mediating M2-type macrophages and/or Th2-type CD4+ T cell responses or activation of mechanisms fostering M1-type inflammation and/or Th1-type immunity is a promising therapeutic strategy against cancer.

MOLECULAR DETERMINANTS OF TAM FUNCTIONS

As mentioned above, TAM generally have phenotype and functions similar to alternative or M2 macrophages [8]. For example, TAM express low levels of the major histocompatibility complex class II and reduced antimicrobial and anti-tumor activity, while increasing production of mediators that promote angiogenesis, such as vascular endothelial growth factor and cyclooxygenase-2 (COX-2)–derived prostaglandin E2, as well as the anti-inflammatory cytokine IL-10 [8]. Another hallmark feature of alternative activation expressed by TAM is the low expression of IL-12 and up-regulated levels of M2-specific genes, such as arginase-1 (Arg-1), macrophage galactose-type C-type lectin–2 (Mgl2), Fizz1, and Ym1 [19]. Recent addition to the molecular repertoire of TAM includes Sema4D and Gas6, which are respectively involved in promoting tumor angiogenesis and cancer cell proliferation [8]. Analysis of the molecular basis of the TAM phenotype has identified the transcriptional factors NF-κB and HIF-1 as master regulators of their transcriptional programs and indicates these factors as central regulators of tumor progression and metastasis [1NF-κB induces several cellular alterations associated with tumorigenesis and more aggressive phenotypes, including: self-sufficiency in growth signals; insensitivity to growth inhibition; resistance to apoptotic signals; immortalization; angiogenesis; tissue invasion and metastasis [20]. Constitutive NF-κB activation often observed in cancer cells may be either promoted by genetic alterations or by microenvironmental signals, including cytokines, hypoxia and reactive oxygen intermediates (ROI) [20]. In particular, proinflammatory cytokines (e.g. IL-1 and TNF), expressed by infiltrating leukocytes, can activate NF-κB in cancer cells and contribute to their proliferation and survival [20]. In addition to target cancer cell functions, NF-κB has been described as a key regulator of inflammation and resolution, whose activation is subject to multiple levels of regulation including inhibitory [20]. New recent evidence indicate that key components of the NF-κB system, including signaling transduction molecules and NF-κB subunits play a central role in the modulation of macrophage functions, as well as TAM activities [21, 22]. Members of the NF-κB/Rel family regulate many genes involved in immunity and inflammation [20]. Two major signaling pathways control the activation of the NF-κB [20]. The classical pathway is stimulated by proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), as well as by recognition of pathogen-associated molecular patterns (PAMPs), and is mostly involved in innate immunity [20]. In addition, an alternative pathway of NF-κB activation, mainly involved in adaptive immunity, is activated by certain members of the TNF cytokine family, but not by TNF-α itself [20]. The NF-κB family consists of five members: NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB and c-Rel, that may form different homo- and hetero-dimers associated with differential regulation of target genes [20]. For example, the p50 and p52 homodimers act as repressors, as these proteins lack a transcription activation domain, present in RelA, RelB, v-Rel and c-Rel [20]. Accumulation of p50 homodimers has been observed in endotoxin tolerant macrophages [22], as well as in TAM [10], suggesting an important role in the control and extinction of the inflammatory response [22]. Other negative regulators of NF-κB activation have been identified in disease and include the LPS-inducible splice variant of myeloid differentiation 88 (MyD88), termed MyD88s, the single immunoglobulin IL-1 receptor-related molecule (SIGIRR)/TIR8, ST2, IRAK-M, SOCS1 and the Src homology 2-containing inositol-5'-phosphatase, SHIP [22]. Exposure to microbial components such as bacterial lipopolysaccharide (LPS) has long been known to induce tolerance to the same agonist in terms of *in vivo* toxicity and macrophage production of inflammatory mediators, such as TNF [23]. This phenomenon has been sometimes referred to as immunoparalysis, which might play a role in sepsis mortality, and it was recently pointed out that in septic patients a reprogramming of the macrophage, rather than an «immunoparalysis», might occur [22].

To the extent they have been investigated, TAM display a defective NF-κB activation in response to different pro-inflammatory signals [10], suggesting their tolerant phenotype. Inhibition of NF-κB activation in TAM correlates with impaired expression of NF-κB-dependent inflammatory functions (e.g. expression of cytotoxic mediators, such as NO, and cytokines (TNFα, IL-1 and IL-12) [3]. Hence, there is an apparent contrast with inflammatory functions expressed by TAM during early steps of carcinogenesis. Possible explanations for this discrepancy are based on the functional feature of macrophages, which are considered highly plastic cells able to finely modulate their programs in response to different microenvironmental conditions [11]. It has been speculated that dynamic changes of the tumor microenvironment may occur during the transition from early neoplastic events toward advanced tumor stages [11]. These events would drive an M1 towards M2 switch of TAM functions (Fig. 2) [11] and are likely connected to the profound changes occurring in the tumor microphysiology (eg. hypoxia, glucose levels, pH). Thus, while full activation of NF-κB in leukocytes would favor M1 inflammation and tumorigenesis, tumor growth and progression may drive inhibition of NF-κB in infiltrating leukocytes, as reported in both myeloid and lymphoid cells associated with advanced tumor stages [11].

Interestingly, TAM from p50-/- tumor-bearing mice express cytokines characteristic of M1 macrophages (eg. IL-12^{high}/IL-10^{low}) and their splenocytes produce increased levels of Th1 cytokines (eg. IFN-γ), which are associated with a delay in tumor growth [10]. By

searching for the microenvironmental signals promoting accumulation of the p50 homodimer in macrophages, we demonstrated that IL-10, PGE_2 and TGF-ß, which are expressed by TAMs and promote M2-type polarized inflammation [23], promote p50 NF-κB homodimer activity [10].

A detailed analysis of the role of p50 NF-κB homodimer in macrophage functions revealed that its nuclear accumulation, both in TAM and LPS-tolerant macrophages, not only mediates a status of unresponsiveness (tolerance) toward pro-inflammatory signals, but actually plays as key regulator of M2-driven inflammatory reactions, acting through inhibition of NF-κB-driven M1-polarizing IFNβ production and STAT1 phosphorylation [22] (Fig. 3).

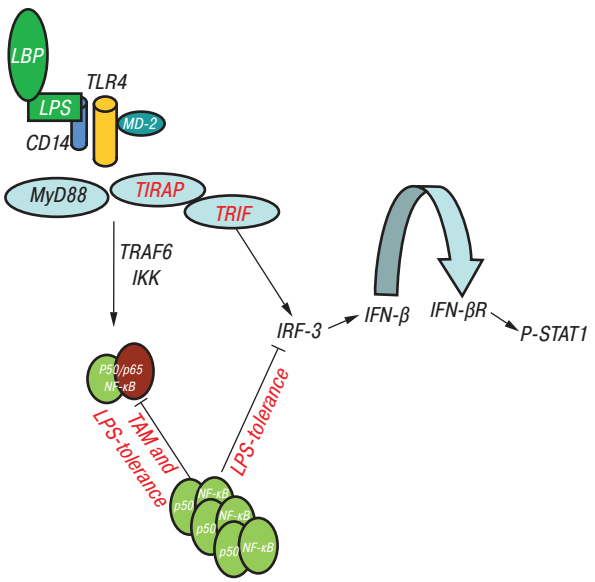

Fig. 3. Different mechanisms of p50 NF-κB-mediated M2 polarization in TAM *vs.* LPS-tolerant macrophages. In LPS tolerance p50 NF-κB acts by suppressing NF-κB driven, M1 polarizing, IFNα/β production, thus preventing STAT1 phosphorylation. In contrast, TAMs have impaired NF-κB activation, but functional STAT1 activity.

A recent study by Hagemann *et al.* [21] demonstrated the requirement for IKKβ to maintain the IL-10^{high}/IL-12^{low} phenotype of TAM in a mouse model of ovarian cancer. These authors showed that targeted deletion or inhibition of IKKβ in TAM increased their anti-tumor activity through elevated NOS2 expression and IL-12–dependent recruitment and activation of NK cells. Because increased expression of IL-12, NOS2, and enhanced anti-tumor activity are associated with M1 characteristics, these data imply that inhibition of the IKKβ/NF-κB pathway promotes an M1-like phenotype in TAM. Additional efforts are required to clarify the role of NF-κB in TAM polarization, but both studies indicate that inhibition of either p50 NF-κB or IKKβ result in restoration of STAT1 activity, which appears a convergent and necessary function to promote M1 macrophage polarization [21, 22]. An early IKKβ dependent NF-κB activation may trigger cancer related inflammation and the p50 dependent regulatory pathway may tune and promote M2 associated smoldering inflammation.

A microenvironmental condition that appears to impact on NF-κB signaling in TAM is hypoxia (low oxygen tension). The presence of many areas of hypoxia is a hallmark feature of most forms of solid tumor [8] and TAM have been shown to accumulate in these areas where hypoxia promotes their pro-tumor phenotype [24]. HIF-1 has been shown to control the cellular response to hypoxia. Hypoxia stabilizes HIF-1α, preventing post-translational hydroxylation and subsequent degradation via the proteasome. More recently, short-term exposure of murine bone marrow–derived macrophages to hypoxia has been shown to up-regulate NF-κB activity, which in turn up-regulates HIF-1α levels [25]. This study used macrophages from $IKK\beta^{-/-}$ mice to show that NF-κB is a critical transcriptional activator of HIF-1α and that basal NF-κB activity is required for HIF-1α protein accumulation under hypoxia. Overall, this evidence indicates a considerable plasticity in NF-κB in TAM and suggests that modulation of its activity in these cells maintains their immunosuppressive, tumor-promoting phenotype. Further studies addressing the relative contribution of individual NF-κB members (p65, c-Rel, p50, BCL3) and their combinatorial transcriptional partners, such as STATs and IRF3 [19], will likely contribute to fully clarify its role in cancer-related inflammation.

THERAPEUTIC MODULATION OF CANCER-RELATED INFLAMMATION

Certain forms of inflammation are protective in a preventive or therapeutic setting [8]. An immune response has long been known to contribute to the outcome of chemotherapy. It has now been shown that dying tumor cells can be cross presented by dendritic cells and trigger a protective immune response via a TLR4-MyD88 pathway [26]. Inflammasome activation and IL-1β production underlie ultimate activation of protective immunity [8]. Association of TLR4 and PrX7 polymorphisms with clinical outcome is consistent with relevance of these pathways.

Direct activation of innate immunity cells is an alternative or complementary strategy [8]. IFNγ has been shown to re-educate TAM and there is proof of principle evidence for antitumor activity of this molecule in minimal residual disease in humans [8]. Given the pro-tumor function of TAM in many cancers, strategies have been directed at blocking recruitment (see above) targeting chemokines or CSF-1 or inhibiting TAM directly [1, 27].

Depleting macrophages using transgenic mouse approaches or the use of pharmacological drugs that knock down macrophages in vivo like clodronate-encapsulated liposomes [3] or amino-bisphosphonate has been shown to reduce angiogenesis and tumor progression in several experimental tumor models [3] suggesting that targeting macrophages presents a potential strategy to control tumor growth. However, as more evidence emerges for the signaling pathways involved in the 'switch' of macrophage polarization states (ie. M1 to M2) in the early stages of tumor progression [11] it may be possible to develop new therapies aimed at preventing this and/or re-orientating M2 TAM in favor of a more anti-tumor phenotype.

In this regard, several lines of evidence support the idea that pharmacological skewing of TAM polarization, from M2 to a full M1 phenotype, may sustain an antitumor activity. Indeed, combination of CpG plus an anti-IL-10 receptor antibody switched infiltrating macrophages from M2 to M1 and triggered innate response debulking large tumors within 16 h [3]. Moreover, TAMs lacking $STAT6^{-/-}$, the major mediator of IL-4 and IL-13 biological functions, display an M1 phenotype, with low level of ARG and high level of NO [8]. As a result, these mice rejected spontaneous mammary carcinoma by a process requiring adaptive immunity to cancer [8].

In analogy, inhibition of STAT3 activity, which is required for IL-10 biological functions and gene transcription, resulted in both restored expression of pro-inflammatory mediators, including IL-12 and TNF-a, by infiltrating leukocytes and tumour inhibition [28].

Recent results suggest that the src homology-2 domain-containing inositol polyphosphate 5'-phosphatase (SHIP) functions in vivo to repress M2 macrophage skewing. $SHIP^{-/-}$ peritoneal and alveolar macrophages constitutively possess high ARG1 and Ym1 levels and impaired LPS-induced NO production. Consistent with this, $SHIP^{-/-}$ mice display enhanced tumor implant growth [8].

A recent work also showed that DNA vaccine against the M2-associated molecule legumain, a member of the asparaginyl endopeptidase family overexpressed by TAMs, induced a robust $CD8^+$ T cell response against TAMs, which reduced their density in tumor tissues and led to a suppression of angiogenesis, tumor growth, and metastasis [8].

All these findings concur to suggest that M2-inflammation may fuel cancer progression and propose a dynamic model of gradual NF-κB inhibition associated with orientation of M2 polarized functions in TAM. It is likely that whereas full activation of NF-κB in inflammatory leukocytes resident in preneoplastic sites may exacerbate local M1-inflammation (TNF-α^{high}, IL-1^{high}, IL-12^{high}, IL-10^{low}, TGFβ^{low}) and favour tumorigenesis [3], tumor growth may result in progressive inhibition of NF-κB in infiltrating leukocytes, as observed in both myeloid [10, 19] and lymphoid [8] cells from tumor bearers, and in the progressive development of M2-inflammation (TNF-α^{low}, IL-1^{low}, IL-12^{low}, IL-10^{high}, TGFβ^{high}). If so, therapeutic efficacy of anti-NF-κB strategies against cancers may be subject to both tumor stage and polarization status of infiltrating leukocytes. In this regard, along with proinflammatry cytokines (eg. TNF-α, IL-1), endogenous ligands of TLRs present into the tumour microenvironment are fibrinogen, HSP and HMGB proteins [8].

CONCLUDING REMARKS

Recent results have highlighted a striking similarity in terms of transcriptional profile, functional properties and underlying transcription factors between TAM and «tolerant» macrophage [23]. Tolerant macrophages belong to the wide spectrum and universe of M2-like polarized phagocytes. Inflammatory reactions, and the activity of macrophages in particular, can exert a dual influence on tumor growth and progression [1, 4]. A functional reprogramming of TAM functions appears to be linked to the dynamic changes of the tumor microenvironment during the transition from early neoplastic events to advanced tumor stages, which would result in progressive modulation of their NF-κB activity and progressive conversion of the TAMs from an M1 to an M2 macrophage phenotype (Fig. 3). Certain chemotherapeutic agents or inflammatory conditions can likewise activate a protective macrophage response. Diversity is a hallmark of cancer related inflammation. Recent results have highlighted how adaptive immunity can orchestrate cancer promoting inflammation and TAM through distinct molecular pathways [8]. Thus, the development of effective strategies to tip the balance by blocking cancer promoting inflammation and/or activating protective innate immunity may require definition of actors at play at different anatomical sites and in different tumors.

REFERENCES

1. **Mantovani A, Allavena P, Sica A, *et al*.** Cancer-related inflammation. Nature 2008; **454**: 436–44.

2. **Mantovani A.** Cancer: inflamming metastasis. Nature 2009, **457**: 36–7.

3. **Sica A, Larghi P, Mancino A, *et al*.** Macrophage polarization in tumour progression. Semin Cancer Biol 2008; **18**: 349–55.

4. **Mantovani A, Sozzani S, Locati M, *et al*.** Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002; **23**: 549–55.

5. **Kim S, Takahashi H, Lin W-W, *et al*.** Carcinoma produced factors activate myeloid cells via TLR2 to stimulate metastasis. Nature 2009; **457**: 102–6.

6. **Kaplan RN, Riba RD, Zacharoulis S, *et al*.** VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 2005; **438**: 820–7.

7. **Erler JT, Bennewith KL, Cox TR, *et al*.** Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. Cancer Cell 2009; **15**: 35–44.

8. **Mantovani A, Sica A.** Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr Opin Immunol 2010; **22**: 231–7.

9. **Fridlender ZG, Sun J, Kim S, *et al*.** Polarization of tumor-associated neutrophil (TAN) phenotype by TGF-beta: «N1» versus «N2» TAN — a new paradigm? Cancer Cell 2009; **16**: 183–94.

10. **Saccani A, Schioppa T, Porta C, *et al*.** p50 nuclear factor-kappaB overexpression in tumor-associated macrophages inhibits M1 inflammatory responses and antitumor resistance. Cancer Res 2006, **66**: 11432–40.

11. **Sica A, Bronte V.** Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest 2007, **117**: 1155–66.

12. **DeNardo DG, Andreu P, Coussens LM.** Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. Cancer Metastasis Rev 2010; **29**: 309–16.

13. **Sinha P, Clements VK, Bunt SK, *et al*.** Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. J Immunol 2007, **179**: 977–83.

14. **de Visser KE, Korets LV, Coussens LM.** *De novo* carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. Cancer Cell 2005, **7**: 411–23.

15. **Mantovani A.** Cancer: inflammation by remote control. Nature 2005, **435**: 752–3.

16. **DeNardo DG, Barreto JB, Andreu P, *et al.*** CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell 2009; **16**: 91–102.

17. **Andreu P, Johansson M, Affara NI, *et al.*** FcRgamma activation regulates inflammation-associated squamous carcinogenesis. Cancer Cell 2010; **17**: 121–34.

18. **Wong SC, Puaux AL, Chittezhath M, *et al.*** Macrophage polarization to a unique phenotype driven by B cells. Eur J Immunol 2009; **182**: 6494–507.

19. **Biswas SK, Gangi L, Paul S, *et al.*** A distinct and unique transcriptional programme expressed by tumor-associated macrophages: defective NF-kB and enhanced IRF-3/STAT1 activation. Blood 2006; **107**: 2112–22.

20. **Karin M.** Nuclear factor-kappaB in cancer development and progression. Nature 2006; **441:** 431–436.

21. **Hagemann T, Lawrence T, McNeish I, *et al.*** «Re-educating» tumor-associated macrophages by targeting NF-kappaB. J Exp Med 2008; **205**: 1261–8.

22. **Porta C, Rimoldi M, Raes G, *et al.*** Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor kappaB. Proc Natl Acad Sci U S A 2009; **106**: 14978–83.

23. **Mantovani A, Sica A, Sozzani S, *et al.*** The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004; **25**: 677–86.

24. **Murdoch C, Muthana M, Coffelt SB, *et al*.** The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer 2008; **8**: 618–31.

25. **Rius J, Guma M, Schachtrup C, *et al.*** NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature 2008; **453**: 807–11.

26. **Apetoh L, Ghiringhelli F, Tesniere A, *et al.*** Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007; **13**: 1050–9.

27. **Pollard JW.** Trophic macrophages in development and disease. Nat Rev Immunol 2009; **9**: 259–70.

28. **Kortylewski M, Kujawski M, Wang T, *et al.*** Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nat Med 2005; **11**: 1314–21.