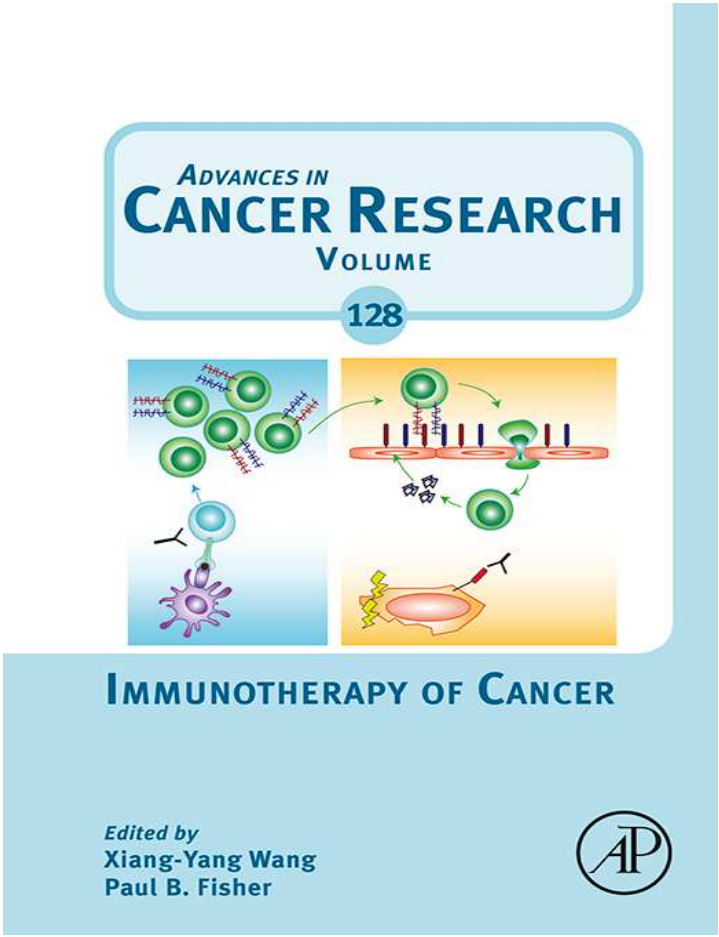

From Eduardo Bonavita, Maria Rosaria Galdiero, Sebastien Jaillon and Alberto Mantovani, Phagocytes as Corrupted Policemen in Cancer-Related Inflammation. In: Xiang-Yang Wang and Paul B. Fisher, editors, *Advances in Cancer Research, Vol. 128*, Burlington: Academic Press, 2015, pp. 141-171.
ISBN: 978-0-12-802316-7

CHAPTER FOUR

Phagocytes as Corrupted Policemen in Cancer-Related Inflammation

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Contents

Abstract

Inflammation is a key component of the tumor microenvironment. Tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs) are prototypic inflammatory cells in cancer-related inflammation. Macrophages provide a first line of resistance against infectious agents but in the ecological niche of cancer behave as corrupted policemen. TAMs promote tumor growth and metastasis by direct interactions with cancer cells, including cancer stem cells, as well as by promoting angiogenesis and tissue remodeling and suppressing effective adaptive immunity. In addition,

[1] E.B., M.R.G., and S.J. have equally contributed to this review and are in alphabetical order. Specifically, E.B. contributed to the field of macrophage and complement and M.R.G. and S.J. to the neutrophils section.

Advances in Cancer Research, Volume 128
ISSN 0065-230X
http://dx.doi.org/10.1016/bs.acr.2015.04.013

the efficacy of chemotherapy, radiotherapy, and checkpoint blockade inhibitors is profoundly affected by regulation of TAMs. In particular, TAMs can protect and rescue tumor cells from cytotoxic therapy by orchestrating a misguided tissue repair response. Following extensive preclinical studies, there is now proof of concept that targeting tumor-promoting macrophages by diverse strategies (e.g., Trabectedin, anti-colony-stimulating factor-1 receptor antibodies) can result in antitumor activity in human cancer and further studies are ongoing. Neutrophils have long been overlooked as a minor component of the tumor microenvironment, but there is evidence for an important role of TANs in tumor progression. Targeting phagocytes (TAMs and TANs) as corrupted policemen in cancer may pave the way to innovative therapeutic strategies complementing cytoreductive therapies and immunotherapy.

1. INTRODUCTION

Epidemiological, genetic, and experimental evidence demonstrate that chronic nonresolving inflammation can increase cancer risk and promotes cancer progression (Coussens, Zitvogel, & Palucka, 2013; Mantovani & Allavena, 2015; Mantovani, Allavena, Sica, & Balkwill, 2008). Tumor-promoting inflammation is now recognized as a key component of cancer (Hanahan & Weinberg, 2011). A link between chronic inflammation and cancer has long been suspected, but only recently the cellular and molecular mechanisms have in part been disclosed.

Epidemiological studies indicate that the risk of carcinogenesis increases under conditions of persistent nonresolving inflammation. Estimate suggests that chronic infections are at the basis of 15–20% of all cancers developed. Examples include viral infections with hepatitis B and C for liver cancer, papilloma virus for cervix carcinoma; bacterial infections, such as *Helicobacter pylori* for gastric cancer or lymphoma; parasites, such as Schistosoma for bladder cancer.

Chronic inflammation can also be triggered by noninfectious agents including irritants such as tobacco smoke, asbestos, silica, gastric reflux, chronic inflammatory disorders of the gastrointestinal tract, and autoimmune diseases can promote cancer development and metabolic dysfunctions, obesity in particular, are associated with a state of low-grade inflammation and increased cancer risk. Long-term use of nonsteroidal antiinflammatory drugs aspirin in particular reduces the risk of carcinogenesis and tumor progression.

Another line of evidence is provided by the composition of the tumor microenvironment, where inflammatory leukocytes and many inflammatory mediators (cytokines, chemokines, enzymes) are present. Inflammatory

cells and mediators are an essential constituent of the tumor microenvironment (Coussens et al., 2013; Hanahan & Weinberg, 2011; Mantovani et al., 2008). Cells of the monocyte–macrophage lineage are major components of the host cell infiltrate of tumors that can reach up to 50% of the total mass. The analysis of the function of leukocyte infiltrate has paved the way to the dissection of tumor-promoting inflammatory mechanisms in cancer (De Palma & Lewis, 2013; Mantovani, Bottazzi, Colotta, Sozzani, & Ruco, 1992; Mantovani, Sozzani, Locati, Allavena, & Sica, 2002; Noy & Pollard, 2014). Indeed, the observation of leukocyte infiltration in tumors was first made by the German pathologist Rudolf Virchow in the nineteenth century, who postulated that cancer may arise in chronically inflamed tissues (Balkwill & Mantovani, 2001; Mantovani et al., 1992).

Not all the tumors have an underlying cause of infection or chronic inflammation, but also in these tumors a reactive inflammatory microenvironment and inflammatory cell infiltration have been described. Inflammation, in these cases, is triggered by the activation of oncogenes (e.g., Ras, Myc, BRAF) and/or the inactivation of tumor-suppressor genes (e.g., p53, PTEN), that in addition to promote cell proliferation, also stimulate the transcription of inflammatory genes, including cytokines and chemokines that recruit circulating leukocytes to the tumor tissues and further fuel the inflammatory response. Several lines of evidence indicate that macrophages have the potential to kill tumor cells and to elicit tumor-destructive reactions. Tumor-associated macrophages (TAMs) are drivers of tumor progression in established tumors, promoting cancer cell proliferation and survival, angiogenesis and lymphoangiogenesis, skewing and taming effective T-cell responses. There is also evidence that inflammatory cells may mediate tumor initiation and promote genetic instability (Mantovani et al., 2008; Noy & Pollard, 2014). Thus, extrinsic causes of inflammation (infections, irritants) and intrinsic causes (oncogene-activated inflammatory response in cancer cells) both concur to build up an inflammatory tumor microenvironment (Mantovani & Allavena, 2015; Mantovani et al., 2008). Here, we will review the role of phagocytes (macrophages and neutrophils) in tumor progression and their connection with humoral innate immunity, prompted by recent evidence (Bonavita et al., 2015).

2. ORIGIN AND FUNCTIONS OF TAMs

Tissue-resident macrophages, characterized in mice by the expression of the chemokine receptor CX3CR1, protect tissues and maintain

homeostasis, whereas inflammatory macrophages, characterized by the expression of CCR2, are recruited at inflammatory sites and contribute to the inflammatory response. Mouse-resident macrophages (Kupffer cells in liver, microglia in brain, Langerhans cells in the skin, and alveolar macrophages in lung) develop in the embryo (Gomez Perdiguero et al., 2015). During this process, progenitors colonize peripheral tissues and differentiate into resident macrophages which will self-maintain throughout life (De Kleer et al., 2014). On the other hand, inflammatory macrophages derive from adult bone marrow-derived monocytes. However, resident macrophages in the gut, heart, and dermis originally derive from the yolk sac, but during adult life are replenished by bone marrow progenitors (Bain et al., 2014; McGovern et al., 2014; Molawi et al., 2014; Wynn et al., 2013). In tumors, TAMs mainly originate from bone marrow monocytes (Franklin et al., 2014; Mantovani et al., 1992; Noy & Pollard, 2014; Shand et al., 2014). In some mouse tumors, local proliferation does occur (Bottazzi et al., 1990; Tymoszuk et al., 2014), but recent evidence suggests that, in general, recruitment of circulating monocytes is essential for TAMs accumulation (Franklin et al., 2014; Noy & Pollard, 2014). Chemokines (e.g., CCL2, CCL5, and CXCL12) and the growth factor CSF-1 (M-CSF) play a major role in monocyte infiltration in tumors. Recently, components of the Complement cascade have also been described to play a role in macrophage recruitment (e.g., Bonavita et al., 2015). Incoming blood monocytes preferentially localize in hypoxic or necrotic areas within tumor stroma; they are profoundly influenced by the tumor environment and rapidly differentiate into tumor-conditioned macrophages. Among chemokines, CCL5/RANTES, CXCL12/SDF-1, and CXC3L1/fractalkine, for instance, were found in neoplastic tissues and contribute to macrophage recruitment and tumor promotion (Balkwill, 2004; Bottazzi et al., 1983; Mantovani et al., 2004; Reed et al., 2012; Ueno et al., 2000). In addition to chemokines and growth factors, noncanonical chemotactic peptides also produced by stromal and tumor cells, such as vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), basic fibroblast growth factor (bFGF), macrophage colony-stimulating factor (M-CSF/CSF-1), urokinase plasminogen activator (uPa), the antimicrobial peptide β-defensin-3, and the lectin Reg3β (Allavena & Mantovani, 2012; Bierie & Moses, 2010; Gironella et al., 2013; Jin et al., 2010; Lin, Gouon-Evans, Nguyen, & Pollard, 2002; Linde et al., 2012; Reed et al., 2012; Zhang, Sud, Mizutani, Gyetko, & Pienta, 2011), caused monocyte recruitment and macrophage differentiation. CXC chemokines (CXCL8, CXCL1,

CXCL2, CXCL3, CXCL5), known for their role in neutrophil recruitment in both physiological and pathological conditions and involved in cancer progression, are also produced in tumor-associated inflammation. This favors tumor angiogenesis and metastasis (Keeley, Mehrad, & Strieter, 2010; Lazennec & Richmond, 2010; Mantovani, Cassatella, Costantini, & Jaillon, 2011).

Plasticity and diversity are key properties of cells of the monocyte–macrophage lineage (Biswas & Mantovani, 2010; Mosser & Edwards, 2008; Sica & Mantovani, 2012). In primary tumors and in metastatic sites, TAMs are involved in complex bidirectional interactions with tumor cells; cancer stem cells (CSCs); fibroblasts; mesenchymal stem cells; endothelial cells; and T, B, and NK cells. Macrophages can undergo polarized classical M1 activation in response to interferon-γ (IFN-γ) and Lipopolysaccharide (LPS), or alternative M2 activation driven by IL-4 or IL-13. M1- and M2-polarized macrophages are extremes of a continuum in a universe of functional states. The molecular mechanisms and functional properties of polarized macrophages have recently been reviewed (Mantovani & Allavena, 2015; Murray et al., 2014). In many mouse and human tumors, TAMs have a frank M2 phenotype or properties which are to some extent shared with M2-polarized cells. In general, TAMs promote tumor growth and metastasis, angiogenesis, and subversion of effective antitumor immunity (Biswas & Mantovani, 2010; Coussens et al., 2013; Sica & Mantovani, 2012). Signals derived from tumors and host cells shape the functional phenotype of TAMs. In different tumor and tissue contexts, these functional determinants include hypoxia, cytokines (e.g., TGF-β and CSF-1), and metabolic products of cancer cells (e.g., lactic acid); IL-4 and IL-13 produced by Th2 cells; and IL-10 produced by Treg cells, B cells, and immune complexes (Colegio et al., 2014; Coussens et al., 2013; De Palma & Lewis, 2013; Mantovani & Allavena, 2015; Mantovani et al., 2008; Noy & Pollard, 2014; Ruffell, Affara, & Coussens, 2012; Sica & Mantovani, 2012). Within the cancer tissue, there can be microanatomical diversity of TAMs function with accumulation of M2-like cells in hypoxic areas (Movahedi et al., 2010). Moreover, inflammatory components and pathways of orchestration differ in tumors originating in distinct anatomical sites (Ruffell et al., 2012).

There is strong evidence that macrophages can be "reprogrammed" by some immunological stimuli, such as IFN-γ or IFN-α, from immunosuppressive M2 macrophages into immunostimulatory cells (De Palma & Lewis, 2013; Duluc et al., 2009). At the clinical level, it has been reported that IFN-γ-driven intratumoral microenvironment exhibits superior

prognostic effect compared with an IFN-α-driven microenvironment in patients with colon carcinoma. This gives a successful proof of principle that complex cytokine interaction networks can be found and dissected in human tissues (Grenz et al., 2013). Moreover, a Th1-dominated tumor micromilieu is strongly associated with a positive prognosis in CRC (Camus et al., 2009; Galon et al., 2006; Naschberger et al., 2008). Several lines of evidence indicate that macrophages infiltrating the tumor take part in the inflammatory process, favoring tumor formation and progression and a M2-like phenotype for TAMs has been reported in several studies (Biswas et al., 2006). The M2-like phenotype can be induced by the tumor cells. Katara et al. reported that vacuolar ATPase (V-ATPase) produced by tumor cells can promote tumor survival and growth. In particular, cancer tissues and cells overexpress the a2 isoform of V-ATPase (a2V). The relevance of the a2V role has been tested in *in vitro* studies, exposing macrophages to the cleaved N-terminal domain of a2V. In these conditions, macrophages express and secrete TAM-associated molecules such as mannose receptor-1, arginase-1, interleukin-10, TGF-β, MMP-9, and VEGF (Katara et al., 2014). A member of the TGF-β family has recently been reported to promote M2-like polarization of TAMs and to inhibit IL-12 (Wang et al., 2014).

During tumor growth and progression, functions of TAMs include extracellular matrix remodeling, promotion of tumor cell invasion and metastasis, angiogenesis, lymphangiogenesis, and immune suppression (Mantovani et al., 2002). In fact, TAMs produce a number of proteolytic molecules, such as plasmin, urokinase-type plasminogen activator, cathepsin B, and matrix metalloproteases (MMPs) which may directly remodel the extra cellular matrix (ECM) (Gocheva et al., 2010; Nagakawa, Aoki, Kasuya, Tsuchida, & Koyanagi, 2002; Wang et al., 2011). The role of MMPs in tumor progression has been suggested by their capacity to degrade the basement membrane to activate growth factors and to enhance angiogenesis (Huang et al., 2002; Stetler-Stevenson & Yu, 2001; Wang, So, Reierstad, & Fishman, 2005). Invasiveness of cancer cells is facilitated by TAMs expression of nonproteolytic molecules. For instance, expression of chemokines that bind CXCR2 was increased in macrophages exposed to conditioned media from mammary epithelial cells containing FGF receptor 1-induced soluble factors. In turn, these chemokines induced migration of primary and tumoral mammary epithelial cells (Bohrer & Schwertfeger, 2012). In mice injected subcutaneously with pancreatic cancer cells, expression of

scavenger-receptor A in hematopoietic cells, consistent with its expression on macrophages, was required for cancer metastasis (Neyen et al., 2013). In glioma stem-like cells, the expression of MMP-9 promoted by macrophages-derived TGF-β1 increased the invasiveness of tumor cells (Ye et al., 2012). Finally, tumor-derived versican V1 enhanced the expression of the antimicrobial peptide hCAP18/LL37 in macrophages, which in turn contributed to ovarian tumor cell proliferation and invasion (Li et al., 2013).

Macrophages have been described to be associated with the metastatic potential of several tumors (Lin, Li, Tadashi, & Dong, 2011; Qing et al., 2012). In classical experiments of Gorelik and coworkers, it was described that transfer of thioglycollate-elicited peritoneal macrophages in mice increased by up to 100-fold the number of metastatic lung nodules induced by the intravenous injection of melanoma or Lewis lung carcinoma tumor cells (Gorelik, Wiltrout, Brunda, Holden, & Herberman, 1982). In a mouse model of breast cancer, IL-4-treated macrophages upregulated the expression of cysteine protease cathepsin B, which promoted lung metastasis (Vasiljeva et al., 2006). Moreover, M2-polarizing cytokines or tumor cell-conditioned media cause macrophages expression of a truncated fibronectin isoform, namely migration-stimulating factor, that is a potent chemotactic factor for tumor cells (Solinas et al., 2010). Depletion studies in experimental animals cause reduced incidence of metastasis, giving further support to the prometastatic function of TAMs (DeNardo et al., 2009; Joyce & Pollard, 2009).

TAMs are associated with tumor angiogenesis and lymphangiogenesis: TAMs express mediators such as TGF-β, VEGF-A, VEGF-C, PDGF, MMP-9, thymidine phosphorylase, and chemokines (e.g., CXCL8/IL-8) which are directly or indirectly involved in new vessel formation and sprouting (Granata et al., 2010; Hotchkiss et al., 2003; Murdoch, Giannoudis, & Lewis, 2004; Schmidt & Carmeliet, 2010; Schoppmann, Horvat, & Birner, 2002). TAMs-derived MMP-9 induces the release of heparin-bound growth factors, particularly VEGF-A, crucial for the angiogenic switch (Ebrahem et al., 2010). VEGF-recruited monocytes improve their performance as angiogenic cells (Avraham-Davidi et al., 2013). Recruited monocytes derive from the pool of circulating Ly6Chi monocytes that undergo phenotypic and functional changes upon entry in the VEGF-rich environment. These recruited monocytes acquire enhanced proangiogenic capabilities and, importantly, a markedly increased capacity to remodel existing blood vessels.

In the tumor microenvironment, low-oxygen tension increases the expression levels of Hypoxia-inducible factor (HIF) -1 and HIF-2, which trigger a proangiogenic program in macrophages characterized by high expression levels of VEGF, bFGF, CXCL8/IL-8, and glycolytic enzymes (Murdoch et al., 2004). In the tumor microenvironment, local hypoxia causes high levels of adenosine that stimulate angiogenic and lymphangiogenic factors released by human (Granata et al., 2010). Casazza and coworkers recently reported that the Sema3A/neuropilin-1 signaling axis controls TAMs localization into hypoxic tumor areas. If TAMs are confined inside normoxic regions by blunting the Sema3A/neuropilin-1 pathway, antitumor immunity is restored and angiogenesis abated, and consequently tumor growth and metastasis are inhibited. Thus, cancer cell-derived Sema3A, not VEGF, is responsible for TAMs entry into hypoxic niches through neuropilin-1 signaling, where TAMs escape antitumor immunity and promote vascularization (Casazza et al., 2013). Modulating TAMs localization and thus their phenotype can be a new approach to guide TAMs activities against cancer. Moreover, Laoui et al. reported that hypoxia is not a major driver of the TAMs subset differentiation found in tumor infiltrate, namely $CD11b^{hi}F4/80^{hi}Ly6C^{lo}$ MHC-II^{lo} or MHC-II^{hi} TAMs, both of which derived from tumor-infiltrating Ly6Chi monocytes, but rather specifically fine-tunes the phenotype of M2-like MHC-II^{lo} TAMs, that as a consequence contain higher mRNA levels for hypoxia-regulated genes than their MHC-II^{hi} counterparts (Laoui et al., 2014).

TAMs also express immunosuppressive potential, secreting or expressing a wide range of molecules, such as TGF-β, iNOS, arginase-1, IDO, and IL-10, known for their immunosuppressive role (Hagemann et al., 2006; Mantovani & Sica, 2010; Sica et al., 2000; Zhao et al., 2012). In murine models of breast cancer, TAMs suppress T-cell functions through their metabolic activities, expressing arginase-1 or iNOS (Bronte & Zanovello, 2005; Chang, Liao, & Kuo, 2001; Doedens et al., 2010; Movahedi et al., 2010). However and particularly in humans, TAMs-mediated T-cell suppression may also occur irrespective of L-arginine metabolism (Kryczek et al., 2006). For instance, TAMs have been shown to express the immunosuppressive molecule B7-H1 in hepatocellular carcinoma (HCC), B7-H4 in ovarian and lung cancer, and B7-H3 in lung cancer (Chen et al., 2012, 2013; Kryczek et al., 2006; Kuang et al., 2009). In addition, TAMs have the capacity to induce the expression of these molecules on cancer cell surface, thus providing a novel mechanism by which cancer cells escape the immune surveillance (Chen et al., 2013).

3. MACROPHAGES IN COMPLEMENT-MEDIATED, PTX3-REGULATED TUMOR PROMOTION

The physiological functions of the Complement system include defence against microbial infections, and disposal of immune complexes and products of inflammatory injury (Ricklin & Lambris, 2013). The Complement system also controls different immunological and inflammatory processes. The latter include enhancement of humoral immunity, regulation of adaptive immunity, apoptotic cell clearance, angiogenesis, cellular regeneration, and growth (Ricklin, Hajishengallis, Yang, & Lambris, 2010). The interaction of Complement components with receptors present on macrophages leads to modulation of cytokine production and induction of inflammatory responses. The myelomonocytic cell lineage expresses Complement receptors which mediate pathogen phagocytosis (e.g., C1qR (s), CR1, CR3, CR4, and CRIg) or induce inflammatory responses (e.g., C3aR, C5aR1, and C5aR2; Bohlson, O'Conner, Hulsebus, Ho, & Fraser, 2014; Fig. 1). C3a and C5a mediate macrophage activation through different signaling mechanisms. For instance, C3a activates NLRP3 inflammasome

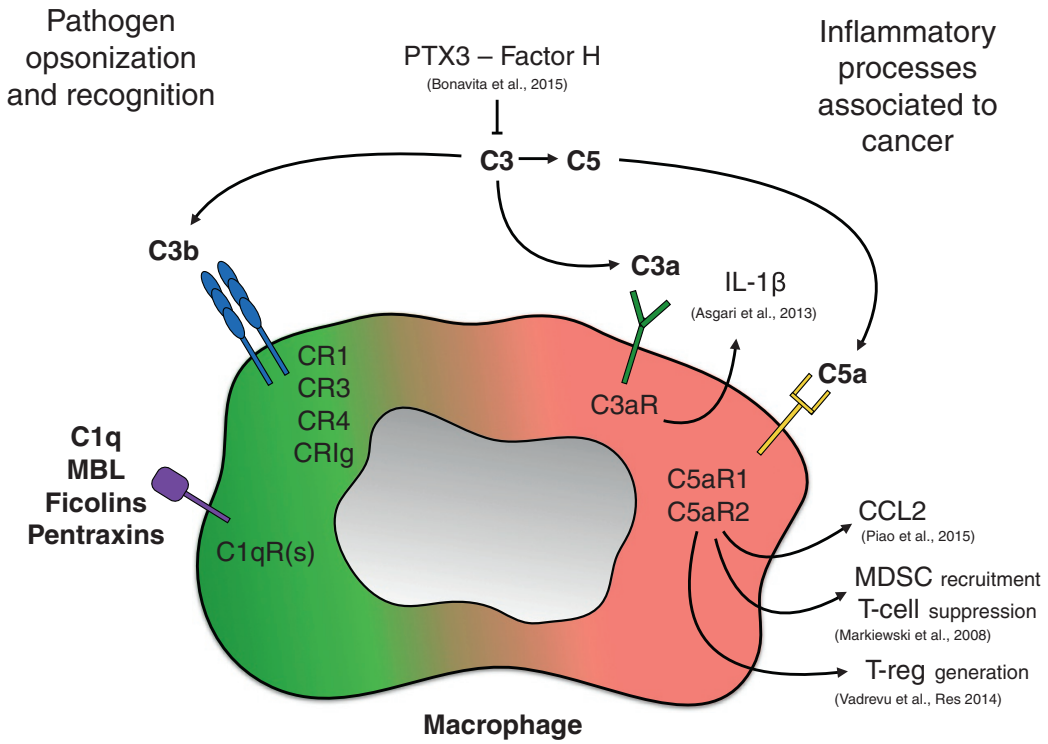

Figure 1 Macrophages and the interplay with humoral innate immunity in the regulation of inflammation and cancer. For explanation, see text.

increasing ATP release and favoring IL-1β production (Asgari et al., 2013). C5a has been correlated with IL-6 induction and development of inflammatory Th17 response (Fang, Zhang, Miwa, & Song, 2009).

During neoplastic transformation, tumor cells can acquire new morphological changes that render them susceptible to Complement attack. The high number of genetic alterations associated with carcinogenesis dramatically changes the composition of the cell membrane. For example, an altered glycosylation is considered a hallmark of cancer cells (Hanahan & Weinberg, 2011), and progression of epithelial cells from a normal to malignant phenotype is associated with an aberrant metabolism of membrane phospholipids affecting signal transduction pathways (Pio, Corrales, & Lambris, 2014). However, in comparison to normal counterpart, tumor cells have also been shown to express higher levels of membrane-bound regulatory proteins and soluble Complement inhibitors, including CD21, CD35, CD46, CD55, CD59, and Factor H, which could be responsible of hindered Complement cytotoxicity (Bellone et al., 2012; Gelderman, Tomlinson, Ross, & Gorter, 2004; Hörl et al., 2013).

Although no formal evidence supports the existence of an effective immune surveillance mediated by Complement during carcinogenesis, changes in the composition of cell membrane may target tumor cells for Complement recognition. Several observations support Complement-mediated recognition of malignant cells and Complement activation in many cancers. Elevated levels of C3a are present in the ascitic fluid of patients with ovarian cancer (Bjorge et al., 2005). C3c and C4 levels are elevated in lung cancer patients and their concentration is directly correlated with tumor volume (Ajona et al., 2013, 2015). The lectin pathway of Complement is more activated in colorectal cancer patients in comparison to healthy individuals, and systemic levels of MASP-2 have been reported to be an independent prognostic marker for poor survival (Ytting, Jarle Christensen, Thiel, Jensenius, & Nielsen, 2005). The activation of the classical pathway of Complement has also been found in patients affected by mucosa-associated lymphoid tissue lymphoma (Bu, Zheng, Wang, & Yu, 2007). Moreover, Complement-dependent cytotoxicity was necessary for immunotherapeutic response to rituximab in central nervous system (CNS) lymphomas (Kadoch et al., 2014) and chronic lymphocytic leukemia (Middleton et al., 2015).

In recent years, many studies have identified new and unexpected roles for Complement activation within the tumor microenvironment challenging the classical view of the Complement system as an anticancer mechanism (Bonavita et al., 2015) Complement elements can promote growth of

transplanted tumors in the context of chronic inflammation (Markiewski et al., 2008). Notably, mice deficient in C3 or C5aR show decreased tumor growth in models of transplantable tumors, in comparison to wild-type mice, suggesting that the Complement system somehow promotes tumor growth (Rutkowski, Sughrue, Kane, Mills, & Parsa, 2010). In line with this view, several studies have demonstrated a protumorigenic role for activated Complement components in all stages of carcinogenesis. The genetic abrogation of C3 significantly reduced tumor incidence in models of 3-methylcholanthrene- and 7,12-dimethylbenz [a] anthracene/terephthalic acid (DMBA/TPA)-induced carcinogenesis (Bonavita et al., 2015). Moreover C3 deficiency was associated with reduced tumor macrophages infiltration (Bonavita et al., 2015). Complement activation promoted azoxymethane/dextran sodium sulphate (AOM/DSS)-induced carcinogenesis in IL-1β/IL-17A-dependent manner and C3-deficient mice developed significantly less colonic lesions (Ning et al., 2015). Complement can suppress antitumoral immunity via C5a that is a potent chemoattractant for myeloid-derived suppressor cells (MDSCs), which inhibit cytotoxic T limphocytes (CTL) (Markiewski et al., 2008). In a preclinical model of breast cancer, C5aR engagement facilitated metastasis by suppressing effectors CD8 and CD4 T-cell responses in the lungs (Vadrevu et al., 2014). In addition, C5a favored liver metastasis by promoting tumor inflammation. Indeed, genetic deficiency of C5aR leads to impaired production of CCL2 (Piao et al., 2015). Finally, data obtained studying pathologies not related to cancer raise the possibility that Complement proteins may enhance Epithelial–mesenchymal transition (EMT), provide chemotactic stimuli (i.e., C5a and C3a; Pasinetti et al., 1996), and induce production of growth factors (i.e., VEGF and TGF-β; Nozaki et al., 2006), which prime and encourage tumor invasion and migration (Christofori, 2006).

Although several lines of evidence sustain a protumoral role for Complement, this system can play different roles in different tumor contexts. For instance, C3 deficiency did not affect tumor incidence in a model of skin carcinogenesis driven by HPV16 (de Visser, Korets, & Coussens, 2004), or even promoted tumor formation in the case of Her2/neu breast tumors (Bandini et al., 2013). Collectively, these data suggest that Complement activation has a dual role in cancer: it has the potential to kill cancer cells, but Complement elements can modulate macrophage functions promoting cancer-related inflammation and tumor progression.

Modulation of Complement activation is a common feature of pentraxins. The short pentraxins C-reactive protein (CRP) and serum amyloid P component recognize different Complement components.

The interaction between CRP and C1q leads to the formation of C3 convertase and thus to the activation of the classical pathway (Sjoberg, Trouw, McGrath, Hack, & Blom, 2006). Surface-bound CRP inhibits alternative pathway amplification through a specific interaction with Factor H, the main soluble regulator of this pathway. In addition, CRP and SAP bind C4b-binding protein (C4BP), a soluble regulator of the classical and lectin pathways (Inforzato et al., 2013).

Similarly, the prototype of long pentraxins PTX3 has a dual role in Complement activation. The first protein identified as PTX3 ligand was C1q, the activator of the classical pathway (Bottazzi et al., 1997). The interaction between PTX3 and the globular head of C1q occurs in a calcium-dependent manner and depending on the way it is presented leads either to activation or inhibition of the Complement cascade (Doni et al., 2012). PTX3 has also been shown to interact with three members of the lectin pathway, namely ficolin-1, ficolin-2, and mannose-binding lectin (MBL; Gout et al., 2011). PTX3 enhances ficolin-1, ficolin-2, and MBL-dependent Complement deposition on the surface of *Aspergillus fumigatus* and *Candida albicans*, respectively, favoring Complement-mediated innate immune responses (Ma et al., 2013). In addition, the formation of a complex ficolin-1/PTX3 on the surface of apoptotic cell facilitated the clearance of apoptotic cells down-regulating in parallel the release of IL-8 by macrophages (Ma et al., 2013). Finally, PTX3 interacts with Factor H, favoring its deposition on PTX3-coated surface and limiting an exacerbated activation of the Complement cascade (Deban et al., 2008). In atypical hemolytic uremic syndrome, mutations observed in Factor H reduced the interaction with PTX3 and lead to enhanced inflammation and Complement-mediated damage (Okemefuna, Nan, Miller, Gor, & Perkins, 2010). PTX3 has also been shown to interact with C4BP, which inhibits Complement activation by acting as a cofactor for factor I in the cleavage and inactivation of C4b (Braunschweig & Jozsi, 2011). This interaction promoted the recruitment of C4BP on late-apoptotic cells and extracellular matrix, suggesting negative modulation of local Complement activation that would otherwise lead to inflammation and tissue damage.

In a model of 3-methylcholanthrene-induced carcinogenesis, PTX3 deficiency was associated to increased susceptibility to cancer, higher proinflammatory mediator release (i.e., CCL2), and gene instability. Tumor tissues from PTX3-deficient mice were characterized by significantly higher C3 deposition in comparison to wild-type tumors because of defective Factor H recruitment. Deficiency of C3 in PTX3 gene-targeted mice was sufficient to rescue the increased susceptibility to tumor growth and

macrophage recruitment. Higher tumor incidence in PTX3-deficient mice was also associated with increased C5a levels and the pharmacological blocking of C5aR *in vivo* reduced tumor frequency (Bonavita et al., 2015). Thus, PTX3 deficiency unleashes unrestrained Complement activation with production of C5a, CCL2, and enhanced recruitment of tumor-promoting macrophages. These results indicate that an essential component of the humoral arm of innate immunity and regulator of Complement activation acts as an extrinsic oncosuppressor by acting at the level of Complement-mediated, macrophage-sustained, tumor-promoting inflammation.

4. THE YIN YANG OF TAMs IN ANTICANCER THERAPY

The evidence and consensus about the role of TAMs in tumor-promoting inflammation (Hanahan & Weinberg, 2011) raise the issue of their involvement in current treatment modalities and of their potential as therapeutic targets. In general, two main approaches have been used: direct depletion of macrophages or inhibition of monocyte recruitment and restimulation of their cytotoxic function (reeducation of TAMs; Beatty et al., 2011; Edwards & Emens, 2010; Germano et al., 2013; Mantovani & Allavena, 2015; Rozel et al., 2009; Xin et al., 2009). As mentioned above, cancer cell-centered therapeutic strategies and immunotherapy profoundly influence the function of TAMs by directly modulating their function or by affecting components of the tumor microenvironment (e.g., effective adaptive immune responses). In turn, TAMs can contribute to the ultimate efficacy of anticancer strategies or retain and amplify their tumor-promoting function by orchestrating a misdirected tissue repair response. The role of TAMs in anticancer therapy has recently been reviewed (Mantovani & Allavena, 2015). Evidence suggests that in conventional cytotoxic therapeutic strategies (chemotherapy and radiotherapy), TAMs can have a dual role. Chemotherapy and radiotherapy can elicit a misdirected macrophage-orchestrated tissue repair response and thus rescue and protect tumor cells including CSCs. On the other hand, TAMs can contribute to the antitumor activity of selected anticancer drugs and low-dose radiotherapy (Mantovani & Allavena, 2015). Moreover, TAMs may play a role in targeted therapies and in checkpoint blockade inhibiting antibodies (Mantovani & Allavena, 2015). Finally, following extensive preclinical testing, there is now proof of principle that targeting TAMs can have antitumor activity in human tumors (Germano et al., 2013). In particular, there is evidence that Trabectedin, approved for clinical use in Europe for sarcomas and ovarian

carcinoma, acts at least in part by depleting tumor-promoting monocytes (Germano et al., 2013).

5. NEUTROPHILS AND CANCER

Neutrophils represent the most abundant leukocyte subpopulation in human peripheral blood and play a primary role in host defence against pathogens during the earliest phases of the inflammatory responses. The role of neutrophils in tumor development has long been underestimated due to their short half-life and terminally differentiated phenotype. In the last decade, the advent of new technical tools allowed to better characterize these cells, thus challenging this limited classical point of view. Indeed, evidences propose emerging roles for neutrophils in coordinating many aspects of the inflammatory response and tumor development. Similarly to TAMs, tumor-associated neutrophils (TANs) can exert both antitumoral and protumoral functions and experimental animal models suggest that neutrophils are characterized by a surprising plasticity (Fridlender et al., 2009; Mantovani, 2009; Fig. 2).

5.1 Neutrophil Recruitment and Their Prognostic Significance in Tumors

Within the tumor microenvironment, a number of CXC chemokines (e.g., CXCL1, CXCL2, CXCL3, CXCL5, CXCL8), known for their neutrophil

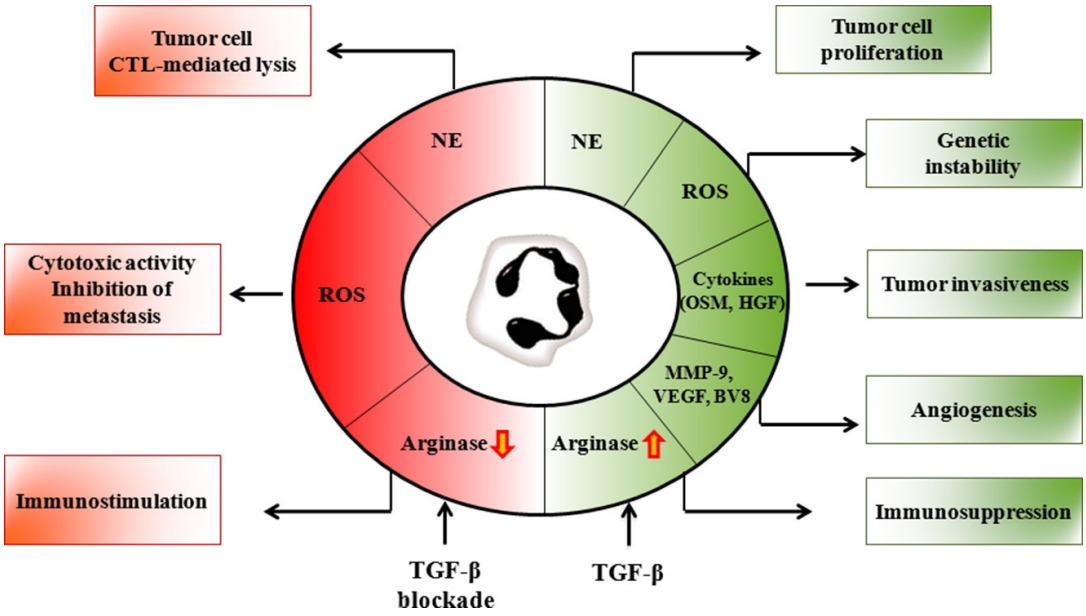

Figure 2 Neutrophils potentially impact key aspects of cancer. For explanation, see text.

chemoattractant properties, are produced by tumor and stromal cells and have been related to cancer initiation, to the promotion of tumor angiogenesis, and metastasis (Keeley et al., 2010; Lazennec & Richmond, 2010; Mantovani et al., 2011). For example, evidence derived from murine models described an important role for the CXCR2 signaling pathway in lung and pancreatic cancer promotion (Ijichi et al., 2011; Keane, Belperio, Xue, Burdick, & Strieter, 2004). In various murine models of cancers (inflammation-associated skin cancer, colitis-associated or spontaneous intestinal cancer), CXCR2 abrogation or neutrophil depletion inhibited both inflammation-induced and spontaneous carcinogenesis (Jamieson et al., 2012). Moreover, in a murine model of graft tumor, CXCL17 promoted the recruitment of myeloid $CD11b^{+}Gr1^{+}F4/80^{-}$ cells within the tumor, favoring tumor growth, angiogenesis, and metastatic behavior (Matsui et al., 2012). In humans, HCC cells and head and neck squamous cell carcinoma (HNSCC) cell lines recruited neutrophils in a CXCR2-dependent manner through the production of CXCL8 (Kuang et al., 2011) and macrophage-inhibiting factor (MIF; Dumitru et al., 2011; Trellakis, Farjah, et al., 2011). Moreover, in a wide cohort of HCC tumors, correlations between increased CXCL5 expression, neutrophil infiltration, and poor patients' survival were found (Zhou et al., 2012). In addition, in a murine model of lung cancer determined by K-ras activation and p53 abrogation, TAM and TAN precursors relocated from the spleen to the tumor and splenectomy significantly reduced the infiltration of myeloid cells within the tumor (Cortez-Retamozo et al., 2012). In addition, Angiotensin II was identified as a pivotal factor in the amplification of hematopoietic self-renewal (Cortez-Retamozo et al., 2013).

Various epidemiological evidences described a negative correlation between TANs and patient clinical outcome in metastatic and localized renal cell carcinoma, bronchioloalveolar carcinoma, HCC, colorectal cancer, and head and neck cancer (Donskov, 2013; Jensen et al., 2009; Kuang et al., 2011; Rao et al., 2012; Trellakis, Bruderek, et al., 2011; Wislez et al., 2003). Moreover, higher tumor-infiltrating neutrophil density was associated with higher histological grade in glioma (Fossati et al., 1999) and more aggressive pancreatic cancer (Reid et al., 2011). In contrast, the association between neutrophil infiltration and patients' clinical outcome remains controversial for some tumor types, such as gastric and colorectal cancer (Caruso et al., 2002; Hirt et al., 2013). These controversial evidences may be due to variability in the methods used to identify neutrophils within tumors (e.g., immunohistochemistry, hematoxylin–eosin staining), as well as the choice of patient datasets and outcomes.

5.2 Neutrophils in Tumor Initiation and Progression

The association between neutrophil-derived reactive oxygen species (ROS) and carcinogenesis has been described already 30 years ago (Weitzman, Weitberg, Clark, & Stossel, 1985). Accordingly, neutrophil-derived ROS and related products, such as myeloperoxidase-mediated HOCl, induced genetic instability, an emerging hallmark of cancer, due to DNA point mutations (Gungor et al., 2010; Hanahan & Weinberg, 2011).

Neutrophil-derived granule proteins can also play dual roles in tumor progression. For instance, neutrophil elastase (NE) can favor tumor cell proliferation via the alteration of the platelet-derived growth factor receptor (PDGFR) intracellular signaling and epithelial-to-mesenchymal transition (Grosse-Steffen et al., 2012; Houghton et al., 2010). In contrast, NE can be taken up by cancer cells, leading to alteration of self-antigens and activation of a CTL-mediated antitumor response (Mittendorf et al., 2012).

Neutrophils also produce a number of cytokines, which play important roles in cancer (Tecchio, Scapini, Pizzolo, & Cassatella, 2013). For instance, stimulated neutrophils secrete Oncostatin M, which stimulates cancer cells to produce VEGF, thus enhancing tumor cell invasive behavior (Queen, Ryan, Holzer, Keller-Peck, & Jorcyk, 2005). In addition, neutrophil-derived hepatocyte growth factor (HGF) promoted the invasive behavior of cholangiocellular and hepatocellular cell lines *in vitro* (Imai et al., 2005). In bronchoalveolar carcinoma patients, an association between neutrophil infiltration, poor patients' prognosis, and levels of HGF in bronchoalveolar lavage fluid was described (Wislez et al., 2003). In HNSCC patients, a correlation between tumor-infiltrating neutrophils and the expression of CORTACTIN, a protein involved in cellular migration, was found (Dumitru et al., 2013). Moreover, tumor-infiltrating neutrophils and CORTACTIN were associated with poor patients' outcome (Dumitru et al., 2013). In contrast, neutrophil-derived molecules can also display antitumoral functions. For instance, neutrophils are an important source of TNF-related apoptosis-inducing ligand (TRAIL), which displays antitumoral activities (Cassatella, 2006; Hewish, Lord, Martin, Cunningham, & Ashworth, 2010). Indeed, *Mycobacterium bovis* Bacillus Calmette–Guerin (BCG) induced the release of TRAIL from neutrophils, suggesting a role for neutrophils in mediating the anticancer effects of BCG in bladder cancer (Kemp et al., 2005). Moreover, neutrophil-derived TRAIL promoted apoptosis of leukemic cells in chronic myeloid leukemia patients (Tanaka, Ito, Kyo, & Kimura, 2007; Tecchio et al., 2004). In

addition, in lung cancer patients, TANs present an activated phenotype, characterized by high expression levels of proinflammatory mediators (i.e., CCL2, CCL3, CXCL8). These activated TANs efficiently stimulate T-cell proliferation and IFN-γ release through a cell contact-dependent manner (Eruslanov et al., 2014). This cross-talk enhanced the expression of costimulatory molecules in neutrophils, sustaining a positive-feedback loop and supporting an antitumoral role for TANs in early stages of human lung cancers (Eruslanov et al., 2014).

5.3 Neutrophils in Tumor Progression: Angiogenesis and Metastatic Behavior Modulation

Neutrophils play a dual role in modulating angiogenesis and metastatic behavior of tumors. Neutrophils express various angiogenic factors, such as VEGF-A, which is also the main mediator of the CXCL1-induced angiogenic activity (Scapini et al., 2004). In murine models of subcutaneous melanoma and fibrosarcoma, in the absence of IFN-β, TANs acquired proangiogenic features, such as increased expression of CXCR4, VEGF-A, and MMP-9 (Jablonska, Leschner, Westphal, Lienenklaus, & Weiss, 2010). MMP-9 is a well-known proangiogenic factor, inducing the release of the active form of VEGF-A from the ECM (Nozawa, Chiu, & Hanahan, 2006).

Bv8 (also known as prokineticin-2) is known to promote neutrophil mobilization and angiogenesis. In a tumor xenograft model, G-CSF induced the expression of Bv8 in neutrophils and blocking Bv8 impaired neutrophil recruitment, tumor growth, and angiogenesis (Shojaei et al., 2007). Interestingly, tumors resistant to anti-VEGF therapy displayed high neutrophil infiltration, and resistance to anti-VEGF treatment was due to G-CSF-induced Bv8 expression. Indeed, blocking G-CSF or Bv8 impaired tumor growth and angiogenesis (Shojaei, Singh, Thompson, & Ferrara, 2008; Shojaei et al., 2009). In contrast, neutrophils also display antiangiogenic properties. For instance, NE itself degraded VEGF and FGF-2 and *in vitro*-generated angiostatin-like fragments from plasminogen, which suppressed VEGF- and FGF-2-mediated angiogenesis (Ai et al., 2007; Scapini et al., 2002). Neutrophils play many roles in modifying the tumor metastatic behavior. Melanoma-derived CXCL8 increased the expression of β_2-integrin on neutrophils, which engaged ICAM-1 expressed on melanoma cells, thus favoring the interaction between neutrophils and melanoma cells. This dangerous interaction allowed melanoma cells to transit across the endothelium,

giving rise to distant metastasis (Huh, Liang, Sharma, Dong, & Robertson, 2010). In addition, neutrophil extracellular traps were able to capture circulating tumor cells and promoted their engraftment to distant organ sites (Cools-Lartigue et al., 2013). In contrast, in an *in vivo* model of breast cancer, under the influence of G-CSF and tumor-derived CCL2, neutrophils accumulated in the premetastatic lung and inhibited metastatic engraftment through the release of H_2O_2. Accordingly, following neutrophil depletion, the metastatic load was significantly enhanced (Granot et al., 2011). Recently, a role for type I IFN signaling in reducing the metastatic load has been described. More in detail, in a model of breast cancer, *Ifnar1*-deficient mice displayed an increased metastatic load together with increased neutrophil infiltration in the premetastatic lung, compared to the wild-type mice. *Ifnar1*$^{-/-}$ neutrophils displayed altered killing activity and increased CXCR2 expression, responsible for their homing in the premetastatic lungs (Wu et al., 2015).

5.4 Neutrophil Plasticity and Heterogeneity in Cancer

In contrast with the classical point of view, neutrophils appear as cells endowed with unsuspected plasticity. In murine models of mesothelioma and lung cancer, neutrophils acquired a protumoral phenotype under the influence of TGF-β (Fridlender et al., 2009). Accordingly, neutrophils recruited in TGF-β-blocking conditions displayed increased antitumor cytotoxic activity, high expression of TNF-α, CCL3, and ICAM-1, and low levels of arginase-1, a well-known T-cell inhibitory factor. TGF-β neutralization also enhanced a T-cell mediated antitumor response, in which neutrophils played a role as effector cells (Fridlender et al., 2009). In contrast, type I interferon signaling has been involved in the acquisition of an antitumoral phenotype in neutrophils. Therefore, in mice lacking type I IFN signals, neutrophils displayed proangiogenic and prometastatic features (Jablonska et al., 2010; Wu et al., 2015). Thus, similarly to the Th1–Th2 and M1–M2 paradigms, a new paradigm has been proposed in which neutrophils can be polarized toward an antitumor N1 or a protumor N2 phenotype in response to signals derived from the microenvironment.

5.5 Neutrophils, TANs, and MDSCs

During cancer development, a heterogeneous population of myeloid cells appears in peripheral blood of tumor-bearing mice and cancer patients. These cells, namely MDSCs, display immunosuppressive and cancer-promoting

properties and are divided into monocytic (Mo-MDSCs) and granulocytic (G-MDSCs) cells, on the basis of distinct morphological and phenotypical aspects (Youn & Gabrilovich, 2010).

The distinction between G-MDSCs and TANs is not so clear. Indeed, neutrophils and G-MDSCs display the same membrane markers (CD11b, Gr1, and Ly6G), similar morphology, and immunosuppressive properties via arginase-1 production (Gabrilovich, Ostrand-Rosenberg, & Bronte, 2012). Accordingly and recently, in a murine model of breast cancer, atypical $CD11b^{+}Ly6G^{+}Rb1^{low}$ neutrophils appeared during tumor progression in peripheral tissues, but not in the primary tumors. This neutrophil subpopulation suppressed T-cell-mediated immune response through the production of ROS. Hematopoietic stem cell differentiation toward the myeloid lineage in bone marrow was found to be driven by tumor-derived G-CSF (Casbon et al., 2015).

In patients with renal cancer, a subset of activated neutrophils in peripheral blood was identified, able to induce T-cell immunosuppression through the production of arginase-1 (Rodriguez et al., 2009; Schmielau & Finn, 2001). Therefore, in this view, these activated neutrophils were considered as G-MDSCs due to their immunosuppressive phenotype. In contrast, MDSCs have been also referred as immature neutrophils (Solito et al., 2011; Trellakis, Farjah, et al., 2011). Indeed, in a genetic conditional lung adenocarcinoma model, TAN precursors physically relocated from spleen to tumors and, since MDSCs accumulated in the spleen of tumor-bearing animals, TAN activities were at least in part attributed to MDSCs (Cortez-Retamozo et al., 2012). Accordingly, G-MDSCs acquired phenotypical and functional aspects of neutrophils, under the influence of GM-CSF, supporting the theory by which G-MDSCs are immature neutrophils (Youn, Collazo, Shalova, Biswas, & Gabrilovich, 2012). Immature neutrophilic MDSCs have also been described in peripheral blood of cancer patients and correlated with poor clinical outcome (Trellakis, Farjah, et al., 2011).

In contrast with these evidences, Fridlender and colleagues performed a transcriptomic analysis on peripheral neutrophils, TANs, and G-MDSCs in tumor-bearing mice, and found that TANs and G-MDSCs are distinct populations of cells and that naïve neutrophils and G-MDSCs are more closely related to each other than to TANs (Fridlender et al., 2012). Accordingly and quite recently, a heterogeneous population of low-density neutrophils (LDNs) has been identified in peripheral blood of tumor-bearing mice and cancer patients (Sagiv et al., 2015). Compared to mature high-density neutrophils (HDNs), LDNs displayed reduced chemotactic activity,

phagocytosis, and oxidative burst as well as lower expression of chemokines (i.e., CXCL1, CXCL2, CXCL10) and chemokine receptors (i.e., CXCR2). From the functional point of view, LDNs impaired $CD8^+$ T-cell proliferation. Thus, in contrast to HDNs, LDNs displayed protumoral activities, which were mainly driven by TGF-β. In addition, within LDNs, two populations of neutrophils were identified, which displayed similar immunosuppressive properties, but different maturation stages. Thus, finally, three distinct populations of neutrophils can be distinguished. The first one consists of HDNs, previously referred as N1 neutrophils, which displayed a mature phenotype together with cytotoxic and antitumor activities. The second and third populations are found within LDNs and consist of immature cells, previously described as G-MDSCs and mature cells, previously described as N2 neutrophils, both sharing immunosuppressive and tumor-promoting functions (Sagiv et al., 2015). Therefore, this increasing body of evidence emphasizes the high versatility of neutrophils in different pathophysiological settings and paves the way for new therapeutic approaches based on their multifaceted biological aspects.

6. CONCLUDING REMARKS

Cells of the myelomonocytic lineage have emerged as a key feature of cancer-related inflammation. They are important players both in the extrinsic pathway connecting inflammation and cancer, consisting of inflammatory conditions which predispose to cancerogenesis, and of the oncogene-driven tumorigenesis process. Macrophage and neutrophils are a major source of humoral fluid-phase pattern recognition molecules such as the long pentraxin PTX3, and their recruitment and function is regulated by the humoral arm of innate immunity. Recent work has highlighted (Bonavita et al., 2015) that Complement and its regulation by PTX3 are an important component of the inflammatory microenvironment and that PTX3 acts as a *bona fide* cancer suppressor gene in mouse and human tumors. There is evidence that targeting TAMs has antitumor activity in human cancer and these preclinical and clinical results are likely to pave the way to innovative therapeutic strategies.

ACKNOWLEDGMENT

Supported by Associazione Italiana per la Ricerca sul Cancro and Italian Ministry of Health.

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