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Macrophage Polarization and Its Role in Cancer

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Abstract

The immune system plays an important role in the development of, and progression of cancer. Macrophages exhibit a variety of responses according to varying stimuli, and express different functions depending upon the microenvironment surrounding them. Macrophages can be pro-inflammatory (M1) or anti-inflammatory (M2). Research studies have shown that infiltration of macrophages can account for >50% of the tumor mass in some cancers, aid in metastasis by inducing angiogenesis, and signify a poor prognosis. Macrophages that migrate to the tumor site, remain there, and aid in angiogenesis and metastasis are termed tumor associated macrophages (TAMs) and are thought to express an M2 phenotype. This review will examine the polarization states of macrophages, their functions and role in cancer, their activation pathways and metabolism, and potential approaches to cancer immunotherapies using macrophages.

Keywords: Macrophage polarization; Inflammation; Cancer

Introduction

Cancer is a group of diseases that involve unregulated cell growth and death, genome instability and mutations, tumor-promoting inflammation, induction of angiogenesis, evasion of the immune system, disregulation of metabolic pathways, immortal cell replication, and activation of metastasis and invasion [1]. Cancer is the second leading cause of death in the United States after heart disease, and more than 1.6 million new cases are expected to be diagnosed each year. More than 580,000 Americans are expected to die yearly from cancer (about 1,600 cancer deaths per day), nearly 1 in 4 of all deaths overall [2,3].

The immune system plays an important role in the development and progression of cancer. In fact, immune cell infiltration to the tumor site can affect malignancy progression and metastasis [4,5]. Infiltration of macrophages to the tumor site has been shown to account for more than 50% of the tumor mass in certain breast cancer cases, suggesting macrophages have a significant role in tumor progression [6-8].

Macrophages are cells derived from the myeloid lineage and belong to the innate immune system. They are derived from blood monocytes that migrate into tissue. One of their main functions is to phagocytose microbes and clear cellular debris. They also play an important role in both the initiation and resolution of inflammation [9,10]. Moreover, macrophages can exhibit different responses depending on the type of stimuli they receive from the surrounding microenvironment, varying from pro-inflammatory to anti-inflammatory [11]. In fact, two major macrophage phenotypes have been proposed: M1 and M2, which correspond to the extreme phenotypes of a spectrum of responses.

The *in vivo* molecular mechanisms of macrophage polarization are poorly characterized because of the variety of signals macrophages experience in the cellular microenvironment [10,12]. In recent years, progress has been made in identifying *in vivo* macrophage polarization under physiological conditions, especially during ontogenesis and pregnancy, and during pathological conditions such as allergies, chronic inflammation, and cancer. However, several macrophage populations are observed during specific pre-clinical and clinical conditions, as a result of the complex signaling between the tissue microenvironment and the immune system [11]. We do know, however, that *in vitro* macrophage polarization is plastic, and macrophages exposed to specific cytokines, can be polarized back and forth to either phenotype [13,14].

This review discusses the characteristics and functions of polarized macrophages, their role in cancer, their activation pathways and metabolic functions, and their use in potential cancer immunotherapy approaches.

M1 Phenotype

M1 macrophages, or classically activated macrophages, are aggressive and highly phagocytic, produce large amounts of reactive oxygen and nitrogen species, and promote a Th1 response [11]. This is a macrophage response usually seen during microbial infections. M1 macrophages secrete high levels of IL-12 and IL-23, two important inflammatory cytokines. IL-12 induces the activation and clonal expansion of Th17 cells, which secrete high amounts of IL-17, and thus contribute to inflammation [15].

In the context of cancer, classically activated macrophages are thought to play an important role in the recognition and destruction of cancer cells, and their presence usually indicates good prognosis. After recognition, malignant cells can be destroyed through several mechanisms, which include contact-dependent phagocytosis and cytotoxicity (i.e. cytokine release such as TNF-α) [16]. Environmental signals such as the tumor microenvironment or tissue-resident cells, however, can polarize M1 macrophages to M2 macrophages. *In vivo*

studies of murine macrophages have shown that macrophages are plastic in their cytokine and surface marker expression and that re-polarizing macrophages to an M1 phenotype in the presence of cancer can help the immune system reject tumors [17].

For a long time, M1 macrophages were thought to be the only functional macrophages and that anti-inflammatory molecules were inhibitory to their function. Now we understand that anti-inflammatory molecules did not inhibit macrophage function but provided and alternative activation of macrophages.

M2 Phenotype

M2 macrophages, or alternatively activated macrophages, are anti-inflammatory and aid in the process of angiogenesis and tissue repair. They express scavenger receptors and produce large quantities of IL-10 and other anti-inflammatory cytokines [18,19]. While they upregulate certain MHC-II molecules, M2 macrophages are not capable of efficient antigen presentation. Expression of IL-10 by M2 macrophages promotes a Th2 response, and Th2 cells, in turn, upregulate the production of IL-3 and IL-4. IL-4 is an important cytokine in the healing process because it contributes to the production of the extracellular matrix [15]. M2 macrophages have different subsets, each induced by a different set of molecules and different activation responses.

M2a macrophages or M(IL-4 or IL-13) are usually referred as profibrotic. They mainly induce a Th2 response and promote type II responses in response to IL-4 and IL-13 [20]. M2b or M(IC or TLR/IL1-R ligands) macrophages are also involved in Th2 activation and immune regulation, and they are often referred as regulators. M2c or M(IL-10 or TGF-β), also described as deactivated, are involved in immune suppression, tissue repair and matrix remodeling. M2d or tumor associated macrophages exhibit functions that may help tumor progression by allowing new blood vessel growth, which feeds the malignant mass of cells, thus promoting their growth [21]. The presence of macrophages (thought to be M2d) in the majority of solid tumors negatively correlates with treatment success and longer survival rates [5].

Anti-inflammatory signals present in the tumor microenvironment such as adiponectin and IL-10, can help macrophages maintain an M2 state and enhance an M2 response [15,22,23].

Macrophage Polarization

The tumor microenvironment significantly affects macrophage polarization. The process of polarization can be diverse and complicated because of the complex environment of IL-10, glucocorticoid hormones, apoptotic cells, and immune complexes that can interfere with the function of innate immune cells [11,17].

Inflammatory signals such as IFN-γ, TNF- α, IL-1ß and LPS can polarize macrophages to the M1 phenotype *in vitro* [24,25]. LPS or IFN-γ can interact with Toll-like receptor 4 (TLR4) on the surface of macrophages inducing the Trif and MyD88 pathways, inducing the activation of transcription factors IRF3, AP-1, and NFκB and thus activating TNFs genes, interferon genes, CXCL10, NOS2, IL-12, etc. which are necessary in a pro-inflammatory response [26]. Classically activated macrophages initiate the induction of the STAT1 transcription factor which targets CXCL9, CXCL10 (also known as IP-10), IFN regulatory factor-1 (IRF-1), and suppressor of cytokine signaling-1 [27].

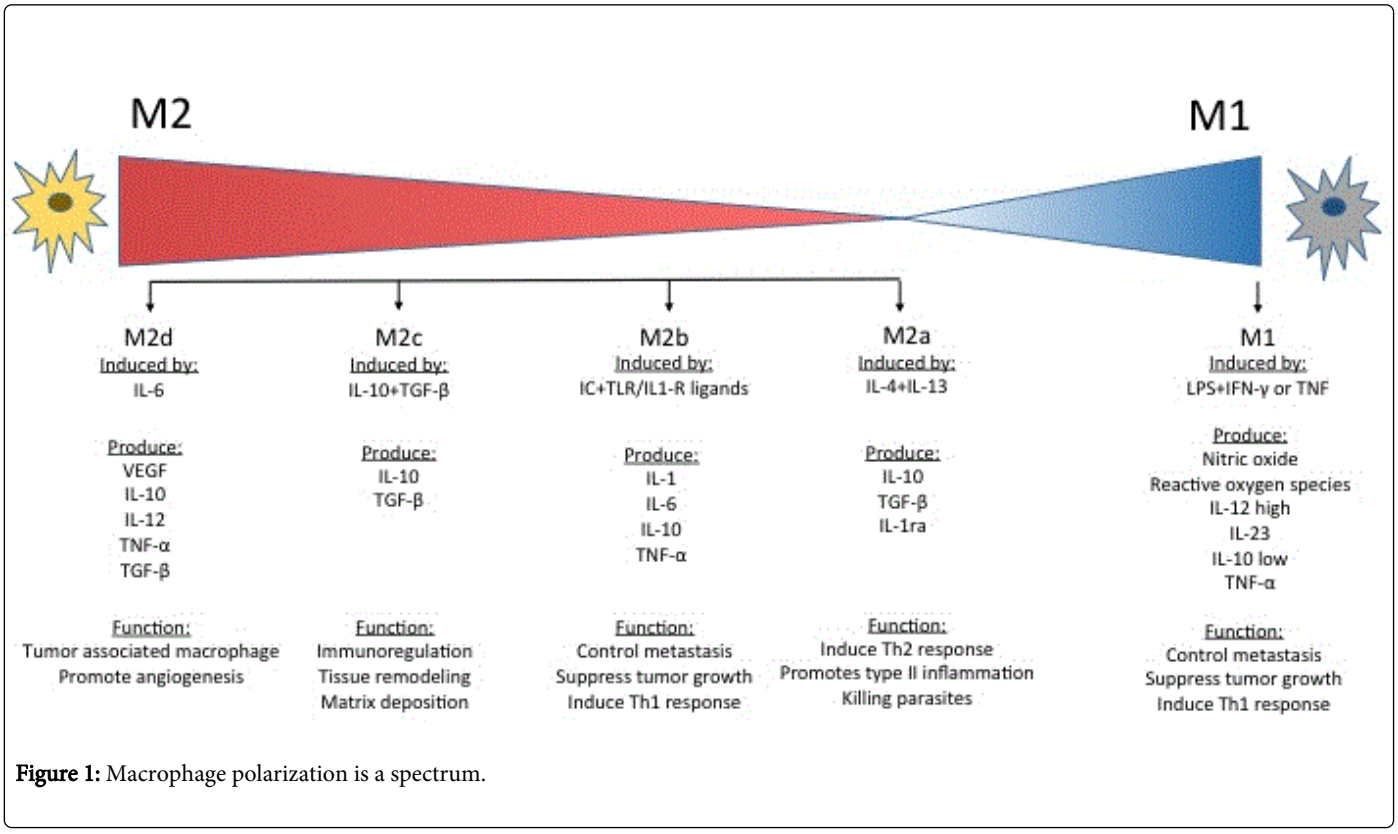

Figure 1: Macrophage polarization is a spectrum.

In the tumor microenvironment, Notch signaling plays an important role in the polarization of M1 macrophages, as it allows transcription factor RBP-J to regulate classical activation. Macrophages that are deficient in Notch signaling express an M2 phenotype regardless of other extrinsic inducers [28]. M2 phenotype subsets are polarized by a variety of signals, which is why it is important to include the inducer molecule when referring to these subtypes (Figure 1) [29].

M2a macrophages are induced by IL-4 and IL-13. IL-4 and IL-13 bind to IL-4R and activate the Jak/Stat6 pathway, which regulates the expression of CCL17, ARG1, IRF4, IL-10, SOCS3, etc., which are genes associated with an anti-inflammatory response. M2b macrophages are usually induced by IC (FCR receptors)+TLR/IL1-R ligands. Additionally, these macrophages over express IL-10, CCL1, IL-1 and IL-6 [30]. M2c macrophages polarize in the presence of IL-10+TGF-β. They upregulate IL-10 and TGF-β production and express CD163 and CD206 as well as several scavenger receptors [21,31].

Other transcription factors associated with macrophage polarization either towards an M1 or M2 response are IRF5, IRF4, Krüppel-like factor 4 (KLF4), CCAAT/enhancer-binding protein-β (C/EBPβ), PU.1, pSTAT1, RBP-J, and CMAF [12,32-35].

Additional mechanisms of macrophage polarization include miRNA micromanagement. miRNAs are small non-coding RNAs of 22 nucleotides in length that regulate gene expression post-transcriptionally, as they affect the rate of mRNA degradation. Several miRNAs have been shown to be highly expressed in polarized macrophages, especially miRNA-155, miRNA-125, miRNA-378 (M1 polarization), and miRNA let-7c, miRNA-9, miRNA-21, miRNA-146, miRNA147, miRNA-187 (M2 polarization) [32,36,37]. miRNA-155, is upregulated when macrophages are transitioning from M2 to M1, and M1 macrophages overexpressing miRNA-155 are generally more aggressive and are associated with reduction of tumors [19]. Moreover, miRNA-342-5p has been found to foster a greater inflammatory response in macrophages by targeting Akt1 in mice. This miRNA also promotes the upregulation of Nos2 and IL-6, both of which act as pro-inflammatory signals for macrophages [38]. Other miRNAs such as miRNA-125 and miRNA-378 have also been shown to be involved in the classical activation pathway of macrophages (M1) [37].

Macrophage polarization is a complex process. In the past years, there has been much controversy on the definition/description of macrophage activation and macrophage polarization. A recent paper published by Murray et al. describes a set of standards to be considered for the consensus definition/description of macrophage activation, polarization, activators, and markers. This publication was much needed to clarify the definition and characterization of activated/polarized macrophages [29]. Basically, the new nomenclature requires to specify the activation molecules (i.e. M(IL-4), M(IL-10), M(LPS), etc.).

Tumor associated macrophages (TAMs)

Cells exposed to a tumor microenvironment behave differently. For example, tumor associated macrophages found in the periphery of solid tumors are thought to help promote tumor growth and metastasis, and have a M2-like phenotype [39]. Tumor associated macrophages can be either tissue resident macrophages or recruited macrophages derived from the bone marrow (macrophages that differentiate from monocytes to macrophages and migrate into tissue). A study by Cortez-Retamozo found that high numbers of TAM precursors in the spleen migrate to the tumor stroma, suggesting this organ as a TAM reservoir. TAM precursors found in the spleen were found to initiate migration through their CCR2 chemokine receptor [40]. Recent studies have found CSF-1 as the primary factor that attracts macrophages to the tumor periphery, and CSF-1 production by cancer cells predicts lower survival rates and indicates an overall poor prognosis [41-43]. Other cytokines such as TNF-α and IL-6 have also been linked to the accumulation/recruitment of macrophages to the tumor pheriphery [42].

It is thought that macrophages that are recruited around the tumor borders are regulated by an "angiogenic switch" that is activated in the tumor. The angiogenic switch is defined as the process by which the tumor develops a high density network of blood vessels that potentially allow the tumor to become metastatic, and are necessary for malignant transition. In a breast cancer mouse model, it was observed that the presence of macrophages was required for a full angiogenic switch. When macrophage maturation, migration, and accumulation around the tumor was delayed, the angiogenic switch was delayed as well, suggesting that the angiogenic switch does not occur in the absence of macrophages, and that macrophage presence is necessary for malignancy progression [44]. Moreover, the tumor stromal cells produce chemokines such as CSF1, CCL2, CCL3, CCL5, and placental growth factor that will recruit macrophages to the tumor surroundings and provide an environment for macrophages to activate the angiogenic switch, during which macrophages will produce high levels of IL-10, TGF-β, ARG-1 and low levels of IL-12, TNF-α, and IL-6. The level of expression of these cytokines suggests macrophages modulate immune evasion. It is important to point out that in solid tumors, macrophages will be attracted by hypoxic environments and will respond by producing hypoxia-inducible factor-1α (HIF-1α) and HIF-2α, which regulate the transcription of genes associated with angiogenesis. During the angiogenic switch, macrophages can also secrete VEGF (stimulated by the NF-κB pathway), which will promote blood vessel maturation and vascular permeability [45].

Tumor associated macrophages are thought to be able to maintain their M2-like phenotype by receiving polarization signals from malignant cells such as IL-1R and MyD88, which are mediated through the IκB kinase β and NF-κB signaling cascades. In fact, inhibition of NF-κB in TAMs promotes classical activation [46]. Moreover, another study suggested p50 NF-κB as the factor involved in suppression of M1 macrophages, and that the inflammation reduction helped in tumor growth. When they created a p50 NF-κB knock-out mouse, they found out that M1 aggressiveness was restored and that tumor survival was reduced [47].

Because the tumor mass contains a great number of M2-like macrophages, TAMs can be used as a target for cancer treatment. Reducing the number of TAMs or polarizing them towards an M1 phenotype can help destroy cancer cells or impair tumor growth [48-50]. Luo et al. in a study published in 2006, used a vaccine against legumain, a cysteine protease and stress protein upregulated in TAMs, as a potential tumor target. When the vaccine against legumain was administered to mice, the results showed that the angiogenesis genes were down-regulated and tumor growth was halted [49].

Metabolism and activation pathways

Metabolic alterations present in tumor cells are controlled by the same mutations that produce cancer [51,52]. As a result of these metabolic alterations, cancer cells are able to produce signals that can

modify the polarization of macrophages and promote tumor growth [53,54].

M1 and M2 macrophages demonstrate distinct metabolic patterns that reflect their dissimilar behaviors [55]. The M1 phenotype is characterized by increased glycolysis and glucose metabolism which is skewed towards the oxidative pentose phosphate pathway, decreased oxygen consumption, and the production of large amounts of radical oxygen and nitrogen species, as well as inflammatory cytokines such as TNF-α, IL-12, and IL-6 [55,56]. The M2 phenotype is marked by increased fatty acid intake and fatty acid oxidation, decreased flux towards the pentose phosphate pathway, increased overall cell redox potential, upregulated scavenger receptors, and immunomodulatory cytokines such as IL-10 and TGF-β [55].

Multiple metabolic pathways play important roles in macrophage polarization. Protein kinases, such as Akt1 and Akt2, alter macrophage polarization by allowing cancer cells to survive, proliferate, and use an intermediary metabolism [57]. Other protein kinases can direct macrophage polarization through glucose metabolism by increasing glycolysis and decreasing oxygen consumption [56,58]. Shu et al. were the first to visualize macrophage metabolism and immune responses *in vivo*, using a PET scan and a glucose analog [59].

L-arginine metabolism also exhibits discrete shifts important to cytokine expression in macrophages and is exemplary of distinct metabolic pathways altering TAM-tumor cell interactions [60]. Classically activated (M1) macrophages favor inducible nitric oxide synthase (iNOS). The iNOS pathway produces cytotoxic nitric oxide (NO), and cells consequently exhibits anti-tumor behavior. Alternatively activated (M2) macrophages have been shown to favor the arginase pathway, and produce ureum and l-ornithine, which contribute to progressive tumor cell growth [60,61].

Direct manipulation of metabolic pathways can alter macrophage polarization. The carbohydrate kinase-like (CARKL) protein, which plays a role in glucose metabolism, has been used to alter macrophage cytokine signatures [55,56]. When CARKL is knocked down by RNAi, macrophages tend to adopt an M1-like metabolic pathway (metabolism skewed towards glycolysis and decreased oxygen consumption), whereas when CARKL is overexpressed, macrophages adopt an M2-like metabolism (decreased glycolytic flux and more oxygen consumption) [55]. When macrophages adopt an M1-like metabolic state through LPS/TLR4 engagement, CARKL levels decrease and the macrophages activate genes controlled by the NFκB pathway such as TNF-α, IL-12, and IL-6, while also increasing cell redox potential by increasing concentrations of NADH:NAD+ and GSH:GSSSG complexes. During an M2-like metabolic state, macrophages upregulate CARKL and genes regulated by STAT6/IL-4 (IL-10 and TGF-β).

Switching the environment to a M1 phenotype bias by targeting metabolic pathways in TAMS may offer an alternative means of reducing tumor growth and metastasis.

Macrophage immunotherapy approaches against cancer

Cancer immunotherapy involves stimulation of the immune system to recognize, reject, and destroy cancer cells. Cancer immunotherapy with macrophages has the goal to polarize macrophages towards a pro-inflammatory response (M1), thus allowing the macrophages and other immune cells to destroy the tumor. Many cytokines and bacterial compounds can achieve this *in vitro*, although the side effects are usually too severe when replicated *in vivo*. The key is to find a compound that will have minimal or easily managed side effects in the patient. Immunotherapy using macrophages has been used in the past decades and new approaches are being developed every year [62,63]. Early immunotherapy established a good foundation for better cancer therapies and increased patient survival rates [64].

Some approaches to cancer immunotherapy are the use of cytokines or chemokines to recruit activated macrophages and other immune cells to the tumor site and allow the tumor cells to be recognized as foreign and destroyed [65,66]. IFN- α and IFN-β have been shown to inhibit tumor progression by inducing differentiation and apoptosis [67]. Also, IFN treatments are anti-proliferative and can increase S phase in the cell cycle [68,69]. Zhang et al. performed a study in nude mice in which they used IFN-β gene therapy to target human prostate cancer cells. Their results show that adenovirus-delivered IFN-β gene therapy involves macrophages and helps suppress growth and metastasis [70].

Another cytokine that can be used in cancer immunotherapy is macrophage inhibitory factor (MIF). MIF is usually found in solid tumors and its presence usually means poor prognosis. MIF, as its name describes, inhibits aggressive macrophage function, and therefore causes macrophages to express an M2 response. An M2 response can aid tumor growth and progression. Simpson, Templeton & Cross (2012) found that MIF induces differentiation of myeloid cells, macrophage precursors, into a suppressive population of myeloid cells that express an M2 response. By targeting anti-MIF shRNA, they were able to deplete this suppressive population of macrophages and inhibit their growth and thus control tumor growth and metastasis [71].

The chemokine receptor type 2, CCR2, is crucial to the recruitment of monocytes to inflammatory sites and it has been shown as a target to prevent the recruitment of macrophages to the tumor site, thus preventing angiogenesis and metastasis. Sanford et al. studied a novel CCR2 inhibitor (PF-04136309) in a pancreatic cancer mouse model, showing that the CCR2 inhibitor depleted monocyte/macrophage recruitment to the tumor site and decreased tumor growth, metastasis, and increased antitumor immunity [72]. Another recent study showed that macrophages co-cultured with 10 different human lung cancers upregulated CCR2 expression. Moreover, they showed that using a CCR2 antagonist in a lung cancer mouse model reduced tumor growth and metastasis [73].

Other studies have used liposomes to deliver drugs to deplete M2 macrophages from tumors and to stop angiogenesis. Cancer cells that express high levels of IL-1β grow faster and induce more angiogenesis *in vivo*. Kimura et al. (2007) found that macrophages exposed to tumor cells expressing IL-1β produced higher levels of angiogenic factors and chemokines such as vascular endothelial growth factor A (VEG-A), IL-8, monocyte chemoattractant protein 1, etc. When they used clodronate liposomes to deplete macrophages, they found fewer IL-1β-producing tumor cells. They also found that by inhibiting NF-κB and AP-1 transcription factors in the cancer cells, tumor growth and angiogenesis were reduced, which may suggest that macrophages found near the tumor site may be involved in the stimuli that promote tumor growth and angiogenesis [74].

Compounds such as methionine enkephalin (MENK) have anti-tumor properties *in vivo* and *in vitro*. MENK has the ability to polarize M2 macrophages to M1 macrophages by down-regulating CD206 and arginase-1 (M2 markers) while upregulating CD64, MHC-II, and the

production of nitric oxide (M1 markers). MENK can also upregulate TNF-α and down-regulate IL-10 [75].

Recent studies have focused on bisphosphonates as a potential inhibitor of M2 macrophages. Bisphosphonates are commonly used to treat metastatic breast cancer patients to prevent skeletal complications such as bone resorption [76]. While bisphosphonates stay in the body for only short periods of time, they can target osteoclasts, cells in the same family as macrophages, due to high affinity for hydroxyapatite. Once bisphosphonates bind to the bones, the bone matrix internalizes the bisphosphonates by endocytosis and once in the cytoplasm, bisphosphonates can inhibit protein prenylation, an event that prevents integrin signaling and endosomal trafficking, forcing the cell to go apoptotic. Until recently, it was unknown whether bisphosphonates could target tumor associated macrophages, but a recent study by Junankar et al. has shown that macrophages uptake nitrogen-containing bisphosphonate compounds by pinocytosis and phagocytosis, events that do not occur in epithelial cells surrounding the tumor [77]. Forcing TAMs to go apoptotic may reduce angiogenesis and metastasis. Another study using bisphosphonates was performed by Coscia et al. where they used zoledronic acid (ZA) to target mammary tumors in mice. They used female BALB-neuT mice that develop spontaneous metastatic breast tumors and administered ZA intravenously once a week for 4 weeks. Compared to control mice (saline injections), ZA-treated mice were able to survive free of cancer for a longer time, they had decreased tumor rates and had a reduction in tumor multiplicity with no toxicity associated with the ZA dosage. When compared to control, the number of infiltrating TAMs was reduced in ZA-treated mice while other lymphocyte numbers remain the same. The authors concluded that ZA impairs TAM recruitment, angiogenesis and VEGF release at the tumor site [78].

Additional approaches to cancer immunotherapy include the use of biomaterials that may elicit an immune response. Cationic polymers are used in immunotherapy because once dissolved in water they can react with nucleic acids, adjuvants, etc. Chen et al. used cationic polymers including PEI, polylysine, cationic dextran and cationic gelatin to produce a strong Th1 immune response. They were also able to induce proliferation of $CD4^+$ cells and to induce secretion of IL-12 in macrophages, a cytokine produced by M1 macrophages [75]. Huang et al. (2013) also used biomaterials to modulate TAMs to an anti-tumor response by targeting TLR4. This study found that TAMs were able to polarize to an M1 phenotype and express IL-12. They found that these cationic molecules have direct tumoricidal activity. They were also able to show tumor reduction in mice (Table 1) [79].

Cancer immunotherapy approaches using macrophages		
Type	**Name**	**Result**
Cytokine/chemokine	IFN-α and IFN-β	Inhibits tumor progression Induce apoptosis in cancer cells Induce differentiation of monocytes to macrophages
	CCR2	Prevents recruitment of monocytes/macrophages to the tumor site
	Anti-MIF shRNA	Depletes M2 macrophage population from tumor site
Inorganic molecules	MENK	Polarized macrophages from M2 to M1
		Downregulates CD2016, arginase 1, and IL-10
		Upregulates CD64, MHC-IL, TNF-α, and nitric oxide
	Bisphosphonates	Induce apoptosis in TAMs
		Reduce the number of infiltrating TAMs to the tumor site
		Impairs angiogenesis
	Cationic polymers (PEI, polylysine, cationic dextran and cationic gelatin)	Induces Th1 response
		Induce proliferation of CD4+ cells
		Induce upregulation of IL-12in macrophages
Vesicles	Liposomes	Deplete IL-1β producing cancer cells
		Inhibits production of angiogenic factor (VEGF-A, IL-8, monocyte chemoattractant protein 1)

Table 1: Summary of cancer immunotherapy approaches.

Conclusion

Macrophages play an important role in tumor progression and metastasis because of the plasticity they express during activation, especially *in vivo*. Depending on the signals present in the tumor microenvironment, macrophages can express pro-inflammatory (M1 phenotype) or anti-inflammatory (M2 phenotype) responses. The tumor microenvironment can polarize macrophages towards an M2 response, an anti-inflammatory response, which can lead to tumor progression, angiogenesis, and metastasis. M2 macrophages resemble tumor associated macrophages (TAMs) which help recruit blood vessels at the tumor site and allow the tumor cells to invade other tissues.

It is obvious that macrophages play a significant role in cancer progression, and immunotherapies involving macrophages should be considered in the treatment of this disease. The polarization of

macrophages towards an M1 response with minimal side effects may prove to be a powerful therapy against solid tumors. Inflammatory signals such as LPS or TNF-α can easily polarize macrophages towards an M1 phenotype *in vitro*. However, use of substances such an LPS and TNF-α *in vivo* exacerbate a whole-body inflammatory response involving cells of both the innate and adaptive immune systems. They can cause fever and inflammation in several tissues including the mucosal surfaces and the lungs. These inflammatory signals are highly cytotoxic as well [80-82]. This can be detrimental to cancer patients and compromise their health.

Current approaches to cancer immunotherapy using macrophages involve cytokines and chemokines and interferons and biomaterials and inorganic molecules that can elicit immune responses. These approaches have been shown to reduce tumor size and angiogenesis, recruit immune cells to the tumor site, and prevent the polarization of macrophages to an M2 phenotype.

Because immunotherapy requires the activation of the immune system, it is difficult to find a cytokine, chemokine, compound, or biomaterial that will not produce some side effects. However, because macrophages belong to the innate immune system and exhibit pro-inflammatory and anti-inflammatory properties, they are ideal immunotherapy candidates.

Further work is needed to identify substances and protocols that can adeptly re-educate the immune system to attack cancer cells, prevent angiogenesis and metastasis, and to protect the host from developing a damaging inflammatory response.

References

1. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646-674.
2. American Cancer Society (2015) Cancer Facts & Figures 201.
3. Hoyert DL, Xu J (2012) Deaths: preliminary data for 2011. Natl Vital Stat Rep 61: 1-51.
4. Kurahara H, Shinchi H, Mataki Y, Maemura K, Noma H, et al. (2011) Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. J Surg Res 167: e211-219.
5. Steidl C, Lee T, Shah SP, Farinha P, Han G, et al. (2010) Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med 362: 875-885.
6. Eiró N, Vizoso FJ (2012) Inflammation and cancer. World J Gastrointest Surg 4: 62-72.
7. Kelly PM, Davison RS, Bliss E, McGee JO (1988) Macrophages in human breast disease: a quantitative immunohistochemical study. Br J Cancer 57: 174-177.
8. Lewis CE, Leek R, Harris A, McGee JO (1995) Cytokine regulation of angiogenesis in breast cancer: the role of tumor-associated macrophages. J Leukoc Biol 57: 747-751.
9. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M (2013) Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol 229: 176-185.
10. Porta C, Rimoldi M, Raes G, Brys L, Ghezzi P, et al. (2009) Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor kappaB. Proceedings of the National Academy of Sciences of the United States of America 106: 14978-14983.
11. Sica A, Mantovani A (2012) Macrophage plasticity and polarization: in vivo veritas. J Clin Invest 122: 787-795.
12. Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, et al. (2011) Krüppel-like factor 4 regulates macrophage polarization. J Clin Invest 121: 2736-2749.
13. Davis MJ, Tsang TM, Qiu Y, Dayrit JK, Freij JB, et al. (2013) Macrophage M1/M2 polarization dynamically adapts to changes in cytokine microenvironments in Cryptococcus neoformans infection. MBio 4: e00264-00213.
14. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A (2002) Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends in Immunology 23: 549–555.
15. Hao NB, Lü MH, Fan YH, Cao YL, Zhang ZR, et al. (2012) Macrophages in tumor microenvironments and the progression of tumors. Clin Dev Immunol 2012: 948098.
16. Sinha P, Clements VK, Ostrand-Rosenberg S (2005) Reduction of myeloid-derived suppressor cells and induction of M1 macrophages facilitate the rejection of established metastatic disease. J Immunol 174: 636-645.
17. Guiducci C, Vicari AP, Sangaletti S, Trinchieri G, Colombo MP (2005) Redirecting in vivo elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection. Cancer Res 65: 3437-3446.
18. Biswas SK, Gangi L, Paul S, Schioppa T, Saccani A, et al. (2006) A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). Blood 107: 2112-2122.
19. Cai X, Yin Y, Li N, Zhu D, Zhang J, et al. (2012) Re-polarization of tumor-associated macrophages to pro-inflammatory M1 macrophages by microRNA-155. J Mol Cell Biol 4: 341-343.
20. Orme J, Mohan C (2012) Macrophage subpopulations in systemic lupus erythematosus. Discov Med 13: 151-158.
21. Chanmee T, Ontong P, Konno K, Itano N (2014) Tumor-associated macrophages as major players in the tumor microenvironment. Cancers (Basel) 6: 1670-1690.
22. Hagemann T, Wilson J, Burke F, Kulbe H, Li N F, et al. (2006) Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype. J Immunol 176: 5023–5032.
23. Mandal P, Pratt BT, Barnes M, McMullen MR, Nagy LE (2011) Molecular mechanism for adiponectin-dependent M2 macrophage polarization: link between the metabolic and innate immune activity of full-length adiponectin. The Journal of Biological Chemistry 286: 13460–13469.
24. Urban JL, Shepard HM, Rothstein JL, Sugarman BJ, Schreiber H (1986) Tumor necrosis factor: a potent effector molecule for tumor cell killing by activated macrophages. Proc Natl Acad Sci U S A 83: 5233-5237.
25. Wong SC, Puaux AL, Chittezhath M, Shalova I, Kajiji TS, et al. (2010) Macrophage polarization to a unique phenotype driven by B cells. Eur J Immunol 40: 2296-2307.
26. Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN (2007) TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med 13: 543-551.
27. Hardison SE, Herrera G, Young ML, Hole CR, Wozniak KL, et al. (2012) Protective immunity against pulmonary cryptococcosis is associated with STAT1-mediated classical macrophage activation. J Immunol 189: 4060–4068.
28. Wang YC, He F, Feng F, Liu XW, Dong GY, et al. (2010) Notch signaling determines the M1 versus M2 polarization of macrophages in antitumor immune responses. Cancer Res 70: 4840-4849.
29. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, et al. (2014) Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 41: 14-20.
30. Özen S, Berk Ö, Şimşek DG, Darcan S (2011) Clinical course of Hashimoto's thyroiditis and effects of levothyroxine therapy on the clinical course of the disease in children and adolescents. J Clin Res Pediatr Endocrinol 3: 192-197.
31. Lu J, Cao Q, Zheng D, Sun Y, Wang C, et al. (2013) Discrete functions of M2a and M2c macrophage subsets determine their relative efficacy in treating chronic kidney disease. Kidney International 84: 745–755.
32. Banerjee S, Xie N, Cui H, Tan Z, Yang S, et al. (2013) MicroRNA let-7c regulates macrophage polarization. J Immunol 190: 6542-6549.

33. Barros MH, Hauck F, Dreyer JH, Kempkes B, Niedobitek G (2013) Macrophage polarisation: an immunohistochemical approach for identifying M1 and M2 macrophages. PLoS One 8: e80908.
34. Hu Y, Zhang H, Lu Y, Bai H, Xu Y, et al. (2011) Class A scavenger receptor attenuates myocardial infarction-induced cardiomyocyte necrosis through suppressing M1 macrophage subset polarization. Basic Research in Cardiology 106: 1311–1328.
35. Lawrence T, Natoli G (2011) Transcriptional regulation of macrophage polarization: enabling diversity with identity. Nat Rev Immunol 11: 750-761.
36. Lodish HF, Zhou B, Liu G, Chen CZ (2008) Micromanagement of the immune system by microRNAs. Nat Rev Immunol 8: 120-130.
37. Squadrito ML, Etzrodt M, De Palma M, Pittet MJ (2013) MicroRNA-mediated control of macrophages and its implications for cancer. Trends Immunol 34: 350-359.
38. Wei Y, Nazari-Jahantigh M, Chan L, Zhu M, Heyll K, et al. (2013) The microRNA-342-5p fosters inflammatory macrophage activation through an Akt1- and microRNA-155-dependent pathway during atherosclerosis. Circulation127: 1609-1619.
39. Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. Nature 454: 436-444.
40. Cortez-Retamozo V, Etzrodt M, Newton A, Rauch PJ, Chudnovskiy A, et al. (2012) Origins of tumor-associated macrophages and neutrophils. Proc Natl Acad Sci U S A 109: 2491-2496.
41. Hercus TR, Thomas D, Guthridge MA, Ekert PG, King-Scott J, et al. (2009) The granulocyte-macrophage colony-stimulating factor receptor: linking its structure to cell signaling and its role in disease. Blood 114: 1289-1298.
42. Smith HO, Stephens ND, Qualls CR, Fligelman T, Wang T, et al. (2013) The clinical significance of inflammatory cytokines in primary cell culture in endometrial carcinoma. Mol Oncol 7: 41-54.
43. West RB, Rubin BP, Miller MA, Subramanian S, Kaygusuz G, et al. (2006) A landscape effect in tenosynovial giant-cell tumor from activation of CSF1 expression by a translocation in a minority of tumor cells. Proc Natl Acad Sci U S A 103: 690-695.
44. Lin EY, Pollard JW (2007) Tumor-associated macrophages press the angiogenic switch in breast cancer. Cancer Res 67: 5064-5066.
45. Dalton HJ, Armaiz-Pena GN, Gonzalez-Villasana V, Lopez-Berestein G, Bar-Eli M, et al. (2014) Monocyte subpopulations in angiogenesis. Cancer Res 74: 1287-1293.
46. Hagemann T, Lawrence T, McNeish I, Charles KA, Kulbe H, et al. (2008) "Re-educating" tumor-associated macrophages by targeting NF-kappaB. J Exp Med 205: 1261-1268.
47. Saccani A, Schioppa T, Porta C, Biswas S K, Nebuloni M, et al. (2006) p50 nuclear factor-kappaB overexpression in tumor-associated macrophages inhibits M1 inflammatory responses and antitumor resistance. Cancer Research 66: 11432-11440.
48. Gazzaniga S, Bravo AI, Guglielmotti A, van Rooijen N, Maschi F, et al. (2007) Targeting tumor-associated macrophages and inhibition of MCP-1 reduce angiogenesis and tumor growth in a human melanoma xenograft. J Invest Dermatol 127: 2031-2041.
49. Luo Y, Zhou H, Krueger J, Kaplan C, Lee SH, et al. (2006) Targeting tumor-associated macrophages as a novel strategy against breast cancer. J Clin Invest 116: 2132-2141.
50. Zeisberger SM, Odermatt B, Marty C, Zehnder-Fjällmana HM, Ballmer-Hofer K, et al. (2006) Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new and highly effective antiangiogenic therapy approach. British Journal of Cancer 95: 272-281.
51. Bettencourt-Dias M, Giet R, Sinka R, Mazumdar A, Lock WG, et al. (2004) Genome-wide survey of protein kinases required for cell cycle progression. Nature 432: 980-987.
52. Shaw RJ (2006) Glucose metabolism and cancer. Curr Opin Cell Biol 18: 598-608.
53. Geschwind JH, Vali M, Wahl R (2006) Effects of 3 ¬ bromopyruvate (hexokinase 2 inhibitor) on glucose uptake in lewis rats using 2-(F-18) fluoro-2-deoxy-d-glucose. In 2006 Gastrointestinal Cancers Symposium (pp. 12–14).
54. Wolf A, Agnihotri S, Micallef J, Mukherjee J, Sabha N, et al. (2011) Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme. J Exp Med 208: 313-326.
55. Blagih J, Jones RG (2012) Polarizing macrophages through reprogramming of glucose metabolism. Cell Metab 15: 793-795.
56. Haschemi A, Kosma P, Gille L, Evans CR, Burant CF, et al. (2012) The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. Cell Metab 15: 813-826.
57. Arranz A, Doxaki C, Vergadi E, Martinez de la TY, Vaporidi K, et al (2012) Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization. Proceedings of the National Academy of Sciences of the United States of America 109: 9517–9522.
58. Jones RG, Thompson CB (2007) Revving the engine: signal transduction fuels T cell activation. Immunity 27: 173-178.
59. Shu CJ, Guo S, Kim YJ, Shelly SM, Nijagal A, et al. (2005) Visualization of a primary anti-tumor immune response by positron emission tomography. Proc Natl Acad Sci U S A 102: 17412-17417.
60. Van Ginderachter JA, Movahedi K, Hassanzadeh Ghassabeh G, Meerschaut S, Beschin A, et al. (2006) Classical and alternative activation of mononuclear phagocytes: picking the best of both worlds for tumor promotion. Immunobiology 211: 487-501.
61. Mills CD, Shearer J, Evans R, Caldwell MD (1992) Macrophage arginine metabolism and the inhibition or stimulation of cancer. J Immunol 149: 2709–2714.
62. Andreesen R, Scheibenbogen C, Brugger W, Krause S, Meerpohl HG, et al (1990) Adoptive transfer of tumor cytotoxic macrophages generated in vitro from circulating blood monocytes: a new approach to cancer immunotherapy. Cancer Research 50: 7450–7456.
63. Korbelik M, Naraparaju VR, Yamamoto N (1997) Macrophage-directed immunotherapy as adjuvant to photodynamic therapy of cancer. Br J Cancer 75: 202-207.
64. Ellem KAO, Rourke MGEO, Johnson GR, Parry G, Misko IS, et al. (1997) A case report: immune responses and clinical course of the first human use of granulocyte/macrophage-colony-stimulating-factor-transduced autologous melanoma cells for immunotherapy. Cancer Immunology, Immunotherapy 44: 10-20.
65. Gast G de, Klümpen H , Vyth-Dreese FA, Kersten MJ, Verra NCV, et al. (2000) Phase I Trial of Combined Immunotherapy with Subcutaneous Granulocyte Macrophage Colony-stimulating Factor, Low-Dose Interleukin 2, and Interferon α in Progressive Metastatic Melanoma and Renal Cell Carcinoma. Clinical Cancer Research 6: 1267-1272.
66. Hill HC, Conway TF Jr, Sabel MS, Jong YS, Mathiowitz E (2002) Immunotherapy with Interleukin 12 and Granulocyte-Macrophage Colony-stimulating Factor-encapsulated Microspheres Coinduction of Innate and Adaptive Antitumor Immunity and Cure of Disseminated Diseases. Cancer Research 62: 7254-7263.
67. Lokshin A, Mayotte JE, Levitt ML (1995) Mechanism of interferon beta-induced squamous differentiation and programmed cell death in human non-small-cell lung cancer cell lines. J Natl Cancer Inst 87: 206-212.
68. Johns TG, Mackay IR, Callister KA, Hertzog PJ, Devenish RJ, et al. (1992) Antiproliferative potencies of interferons on melanoma cell lines and xenografts: higher efficacy of interferon beta. J Natl Cancer Inst 84: 1185-1190.
69. Qin XQ, Runkel L, Deck C, DeDios C, Barsoum J (1997) Interferon-beta induces S phase accumulation selectively in human transformed cells. J Interferon Cytokine Res 17: 355-367.
70. Zhang F, Lu W, Dong Z (2002) Tumor-infiltrating macrophages are involved in suppressing growth and metastasis of human prostate cancer cells by INF-beta gene therapy in nude mice. Clin Cancer Res 8: 2942-2951.
71. Simpson KD, Templeton DJ, Cross JV (2012) Macrophage Migration Inhibitory Factor Promotes Tumor Growth and Metastasis by Inducing Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. J Immunol 189: 5533-5540.

72. Sanford DE, Belt BA, Panni RZ, Mayer A, Deshpande AD, et al. (2013) Inflammatory monocyte mobilization decreases patient survival in pancreatic cancer: a role for targeting the CCL2/CCR2 axis. Clinical Cancer Research 19: 3404-3415.
73. Schmall A, Al-Tamari HM, Herold S, Kampschulte M, Weigert A, et al. (2015) Macrophage and cancer cell cross-talk via CCR2 and CX3CR1 is a fundamental mechanism driving lung cancer. Am J Respir Crit Care Med 191: 437-447.
74. Kimura YN, Watari K, Fotovati A, Hosoi F, Yasumoto K, et al. (2007) Inflammatory stimuli from macrophages and cancer cells synergistically promote tumor growth and angiogenesis. Cancer Sci 98: 2009-2018.
75. Chen H, Li P, Yin Y, Cai X, Huang Z, et al. (2010) The promotion of type 1 T helper cell responses to cationic polymers in vivo via toll-like receptor-4 mediated IL-12 secretion. Biomaterials 31: 8172-8180.
76. Rogers TL, Holen I (2011) Tumour macrophages as potential targets of bisphosphonates. J Transl Med 9: 177.
77. Junankar S, Shay G, Jurczyluk J, Ali N, Down J, et al. (2015) Real-time intravital imaging establishes tumor-associated macrophages as the extraskeletal target of bisphosphonate action in cancer. Cancer Discov 5: 35-42.
78. Coscia M, Quaglino E, Iezzi M, Curcio C, Pantaleoni F, et al. (2010) Zoledronic acid repolarizes tumour-associated macrophages and inhibits mammary carcinogenesis by targeting the mevalonate pathway. Journal of Cellular and Molecular Medicine 14: 2803-2815.
79. Huang Z, Yang Y, Jiang Y, Shao J, Sun X, et al. (2013) Anti-tumor immune responses of tumor-associated macrophages via toll-like receptor 4 triggered by cationic polymers. Biomaterials 34: 746-755.
80. Apostolaki M, Armaka M, Victoratos P, Kollias G (2010) Cellular Mechanisms of TNF Function in Models of Inflammation and Autoimmunity. TNF Pathophysiology. Molecular and Cellular Mechanisms 11: 1-26.
81. Kolb WP, Granger GA (1968) Lymphocyte in vitro cytotoxicity: characterization of human lymphotoxin. Proc Natl Acad Sci U S A, 1250–1255.
82. Michel O, Nagy AM, Schroeven M, Duchateau J, Nève J, et al. (1997) Dose-response relationship to inhaled endotoxin in normal subjects. Am J Respir Crit Care Med 156: 1157-1164.

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