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Nanopolymer-Mediated Targeting Cancer with M1 Macrophage Polarizing Factors: A Unique Strategy to Fight against Cancer

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ABSTRACT

Macrophages are abundantly present, approx. 1010 cells, in almost all tissues of humans. They mainly function as phagocytic cells to make the human body disease-free from any eternal pathogens. The main two types of macrophages, M1 and M2 are known, of which M1 macrophages produces toxic NO, citrulline, tumor necrosis factor-α (TNF-α), and other pro-inflammatory cytokines, IL-12, IL- 23. M1 type while known as pro-inflammatory and also for their microbicidal and tumoricidal activities, the other type, M2 macrophages, are found to be anti-inflammatory but tumor-promoting, one.

The unique features of M1 and M2 macrophages either as a tumoricidal or as a tumor promoting could be interested as a mechanism of tumor therapy, in future. Here, we review the strategies to exploit macrophages as therapeutic tools and targets in cancer therapy. In particular, the role of tumor-associated macrophages (TAMs) in cancer therapy will be focused.

Keywords

Macrophages, Cancer cells.

Abbreviations

Arg-NPs: Arginine nanoparticles, β2m: β2-microglobulin, CTL: Cytotoxic T lymphocytes, MHC-II: Histocompatibility complex II class, INF: Interferon, IL-2: Interleukin-2, IL-12: Interleukin-12, JNK: C-Jun N-terminal kinase, LILRB-1: Leukocyte immunoglobulin-like receptor-1, LPS: Lipopolysaccharides, MAPK-JNK: mitogen-activated protein kinase/c-Jun NH2-terminal kinase, MHC: Major histocompatibility complex, NO: Nitric oxide, RIP-1: Receptor-interacting protein-1, TRAF-2: TNF receptor-associated factor-2, ROS: Reactive oxygen species, SIRP-α: Signal-regulatory protein-α, Siglec-10: Sialic-acid-binding Ig-like lectin-10, TNF-α: Tumor necrosis factor-α, TAMs: Tumor-associated macrophages, TFNR-1: TNF-α tumor necrosis factor receptor-1, TRADD: TFNR-1 associated with DEATH domain protein, Th-1/-2: T helper-1 /-2, TAM: Tumor associated macrophages.

Introduction

Macrophages are motile cells, can enter the site of injury with high destructive potential. In addition to killing pathogens and any foreign cells, macrophages are capable in presenting the main histocompatibility complex II class (MHC II) antigen [1]. Current cancer treatment strategies are not only includes radiotherapy, chemotherapy, or surgical re-section as but also opened up the era of targeted as well as immunotherapy. Modulation of TAMs by polarizing to M1 and activating their signaling system has emerged as a promising and novel immunotherapy for cancer therapy.

Cell-to-cell contact is the manner in which activated macrophages attack cancer cells [2,3], at the time activated macrophages attack cancer cells, the concentrations of chitotriosidase and protease on cell surface will be high enough to induce cancer cell lysis. A number of serine proteases, such as elastase, collagenase and plasminogen activator, are synthesized and secreted by activated macrophages, similar to chitotriosidases [4-7].

How Do Macrophages Recognize and Kill Cancer Cells? (Figure 1)

- In fact, more than 50% tumor associated cells are macrophages [8].
- Macrophages can sense the differences of tumor cell-

membrane composition than normal cell membranes. One of such tumor markers include an increased content of phosphatidylserine. Other may include the altered glycan structures (or glycosylation), like, carcino-embryonic antigen Tn antigen on the tumor cell surfaces, which could be recognized by lectin-like receptors on the cell membranes of macrophages [9].

- Currently, the molecular mechanisms of the antitumor activity of M1-type macrophages are not fully understood.
- It is known that macrophages can kill cancer cells by several mechanisms, such as:
  1. Indirect killing by recruitment of other immune cells that can lyse the cancer cells,
  2. Cytolysis of cancer cells through antibody (Ab) dependent cellular cytotoxicity, and
  3. M1 macrophages can kill target cells directly by producing nitrosative/oxidative stress (NO/ROS), which induces DNA damage, cytotoxicity, and apoptosis [10-13].
- M1 macrophages are known to promote also indirect cytotoxicity by activating other immune cells, such as NK cells and T cells [14-16].
- The innate immune receptor Dectin-1 is expressed on dendritic cells and macrophages which is critical to NK-mediated killing of tumor cells that express N-glycan structures at high levels [17].

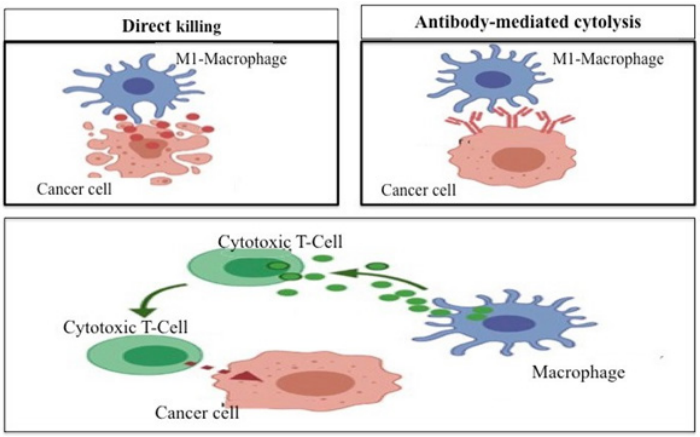


Figure 1: Mechanisms of macrophage-mediate cancer cell killing: 1. Direct killing; 2. Cytolysisi through antibody dependent cellular cytotoxicity; 3. Indirect killing.

How to Stimulate M1-type of Macrophages in Cancer Cells

- In the T helper 1 (Th1) based response, macrophages undergo M1-type activation, synthesizing chitotriosidases, protease, NO, H_2O_2, and other chemicals that kill invaders, such as fungi, viruses, and bacteria.
- Th1 response also can destroy Cancer cells. In this case interferon (INF), interleukin12 (IL12), IL2, and tumor necrosis factor (TNF) play major roles [18,19].
  - TNF-α tumor necrosis factor receptor 1 (TFNR1) signaling pathway [20].
  - TNF-α binds to its receptor TFNR1 associated with DEATH domain protein (TRADD), receptor-interacting protein 1 (RIP1), and TNF receptor-associated factor 2 (TRAF2) to form Complex II.
  - Complex II induces ROS production and activation of caspase-3 and caspase-7 [21,22], and lyse the cancer cells.
  - TNF-α also induces cancer cell apoptosis through the MAPK-JNK pathway [21].

- However, In the T helper 2 (Th2) response, IL4 and IL10 play major roles, and macrophages undergo M2 type activation [18,19]. The mechanisms that control the immune response are not yet known. However, dendritic cells [23], $CD4^+$/$CD25^+$ regulatory T cells [24-26], and T helper17 cells [27] are involved.

Because only the activated M1 macrophages produce 50kDa chitotriosidases, we need to convert the Th2 response to the Th1 response in patients with cancer. To successfully induce the Th1 response, three points need to be followed.

- The amount of the stimulator used to stimulate the Th1 response must be appropriately low, otherwise resistant may develop [28,29].
- Either below or higher of that special range, naive T cells would develop the Th2 response and produce a large amount of IL4 *and little IFN-y* [30].

Polarization of Tams and their Role in the TME

- Theoretically, TAM could be differentiated into M1 phenotype macrophages by Th1 (IFN-γ, TNFα, and LPS et al.), and into M2 by Th2 (IL-4, IL-10, TGFβ1, and PGE2 et al.) cytokines [31] (Figure 2).
- M1 macrophages are characterized by the expression of HLA-DR and CD197, whereas M2 typically express scavenger receptor (CD163), CD209, mannose receptors (CD206), and CCL2, VEGF, and IL-10, etc. [32,33].
- A few sunsets of TAMs in the TME express CD86 and CD80 markers and are termed as M1-like TAMs, and typically exhibit anti-tumor effects [34].

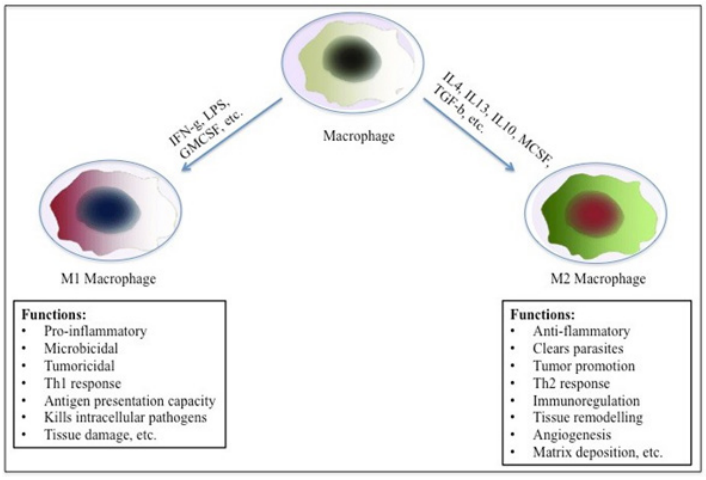


Figure 2: Polarization of TAMS and their role in the TME.

Cancer Treatment Strategy: Targeting Tam Repolarization to M1 Type

Targeting TAMs for cancer treatment has two main directions:

- To prevent macrophage recruitment to the TME; and
- To regulate TAM repolarization [35].
- Restriction of the infiltration of TAMs by blocking the CSF-1/ CSF-1 receptor and CCL2/CCR2 pathways showed some success, also.

A: STAT Signaling Pathways Drives M1 Polarization and cancer inhibition:

- The mechanisms of TAM polarization are found to be correlated with several signaling pathways that includes C-Jun N-terminal kinase (JNK), PI3K/ Akt, JAK/ STAT and Notch signaling pathways [36].
- The JAK/STAT1/STAT6 signaling pathway can be activated by IFN-g [37], which can induce NAMPT-driven glycolysis, M1 polarization.
  - Inhibition of TAM-derived IL-6 can modulate CCL2 secretion, thus inhibits $CD163^{+}$ TAM polarization in colorectal cancer and attenuates tumor occurrence [38].

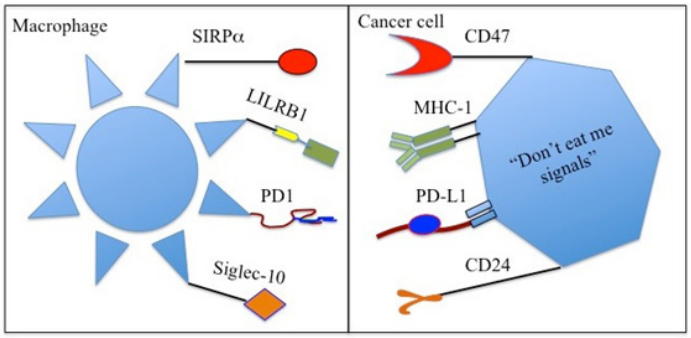
B: Inhibition of PI3Kg /AKT Signaling Pathway drives M1 Polarization and cancer inhibition

- PI3K inhibitor, LY294002 effectively suppresses the polarization of M2 macrophages, and thereby their tumorigenic process [39].
- Further, the PI3Kg pathway-blocking drug BEZ successfully switched TAMs from M2 to M1 phenotype and inhibits pancreatic cancer progression [40].

C: Inhibition of CD47/SIRPa Signaling Pathway can stimulate M1 Polarization

- The CD47/SIRPa *"do not eat me"* signal is of great interest in terms of the anti-phagocytic ability of macrophages.
- Anti-CD47 treatment has been reported to regulate the transformation of M2-like TAMs into the M1 phenotype *in vivo* [41].

Targeting the CD47/SIRPa axis results better prognosis in various cancers such as ovarian, breast and colorectal cancer [42] (Figure 3).



SIRP α: Signal regulatory protein- α
LILRB1 Gene - Leukocyte Immunoglobulin Like Receptor B1
PD1: Programmed cell death protein
Siglec-10: Sialic acid binding Ig-like lectin 10

Figure 3: Anti-phagocytic checkpoints in the tumor microenvironment. The expression of "don't-eat-me" signals on tumor cells, including, CD47, PD-L1, MHC-1, and CD24, protect tumor cells from phagocytic clearance by interacting with their cognate receptors on macrophages.

D: Other Factors Involved in Macrophage Polarization

- Lactic Acid and Tumor Acidosis Promotes M2 Macrophage Polarization and thus tumor invasion [43,44].
  - Whereas, M1 pro-inflammatory macrophages rely mainly on glycolysis, which in turn increases lactic acid levels [43].
  - Therefore, it is crucial to regulate the balance between lactate levels and the degree of tumor acidosis in the TME to inhibit immunosuppression.
- **Iron Promotes M1 Macrophage Polarization**
  - Iron facilitates M1 polarization, while inhibits M2 activation, and exerting tumor immunotherapy effects.
  - Further, Fe level can up-regulate the expression of CCL2, IL-1b, TNF-a, and IL-6, and thereby activates the NF-kB signaling pathway, and finally the M1/M2 macrophage polarization [45].
- **Phytochemicals and Macrophages Polarization**
  - Curcumin blocks M2 polarization in microglial cells of the mouse brain and increase the M1 polarization by inducing STAT1 and NF-kB signaling pathways [46].

E: Targeting Macrophage-Derived and Cancer Cell-Derived Exosomes

- Exosomes are typically between 30 and 150 nm in diameter in size, enclosed by a lipid membrane, which have been found to participate in the interaction between tumor cells and macrophages.
- Both, macrophage-derived and cancer cell-derived exosomes are associated with various signaling pathways that control the tumor immune evasion, metastasis, and drug resistance [47].
- For example, exosomal miR-138-5p derived from breast cancer cells suppresses the expression of KDM6B in macrophages and inhibits M1- related gene expression and thereby promoting breast cancer metastasis [48].
- Therefore, corrections of the ability of exosomes to target tumors and to overcome the biological barriers, like blood–brain barrier and gastrointestinal tract, are emerging as potential therapeutic strategies [47].

F: M2 macrophages are capable of active proliferation *in situ*

- They express higher levels of several marker receptors including:
  - CD36 scavenger receptor for apoptotic cells,
  - CD206 mannose receptor,
  - CD301 receptor for galactose, and N-acetylglucosamine,
  - CD163 receptor for the hemoglobin–haptoglobin complex.
- Macrophages of this type are characterized by a low IL-12/ IL-10 secretion ratio.
- Furthermore, M2 phenotype macrophages are subdivided into M2a, M2b, M2c, and M2d subtypes depending on their markers and functions [49-53].

- The possibility of transformation of the M1 phenotype into M2 was shown with a range of cytokines and as a result of efferocytosis.
- The reverse transformation of the M2 phenotype into M1 is assumed with the development of obesity [54-56].

How Do Tumor Cells Bypass the Antitumor Effect of Macrophages?

- Although macrophages exhibit tumoricidal and phagocytic activity of tumor cells *in vitro,* in reality, tumor cells can bypass the immune system and avoid the action of macrophages.
- CD47, PD-L1, major histocompatibility complex (MHC) class I, and CD24 are the molecules that are expressed by tumor cells and can inhibit the phagocytic activity of macrophages.
- In fact, signal-regulatory protein-α (SIRPα), PD-1, leukocyte immunoglobulin-like receptor 1 (LILRB1), and sialic-acid-binding Ig-like lectin-10 (Siglec-10), the respective binding receptors-45 are expressed on macrophage surface (Figure 3).
- **CD47:** The cancer cells often over-express the membrane-bound protein CD47, which is often called the *"don't-eat-me"* signal. This signal suppresses the phagocytic activity of macrophages upon binding to SIRPα (signal regulatory protein α)-receptor present on phagocytes [57-59].
- Blocking CD47-SIRPα binding promotes phagocytosis of tumor cells by macrophages and induces antitumor responses in different xenograft models [57,60].
- **MHC1:** In addition, a second mechanism was found to play by the MHC class I component β2-microglobulin (β2m) that are expressed by cancer cells which can protect them from phagocytosis. Disruption of either MHC class I potentiates phagocytosis of tumor cells both *in vitro* and *in vivo* [61].
- **PD-1:** PD-1 is a membrane protein of the immunoglobulin superfamily that is involved in the cellular differentiation of immune cells. PD-1 and its ligands, PD-L1 and PD-L2, prevent the activation of T lymphocytes [10-13].

More recently, PD-1 has been found in TAMs, and its expression correlates with tumor growth [62]. PD-1$^-$ macrophages exhibit a higher level of phagocytic activity compared to PD-1$^+$ macrophages. Further, blockade of PD-1 or PD-L1 with Abs leads to increased phagocytosis of tumor cells and suppresses tumor growth. Thus, the PD-1 not only serves as a checkpoint for T cells but also contributes to the evasion of tumor cells from the killing by macrophages.

- **CD24:** Some tumor cells overexpress a glycosylated surface protein, CD24, which interacts with Siglec-10 on immune cells, and suppress inflammatory responses caused by tissue damage and avoid phagocytosis by macrophages expressing Siglec-10 [63-65].
- **Siglec-10:** Tumor-associated M2 macrophages express higher levels of Siglec-10 and are less phagocytic than M1 macrophages.

Use of Biologically Active Compounds for Macrophage Activation, Polarization, and Reprogramming

- **LPS:** It is well known that bacterial LPS can activate macrophages via Toll- like receptors [66]. However, the use of LPS for the activation and polarization of macrophages for anticancer immunotherapy in patients is practically underscored due to the detrimental effect of LPS on the immune system. In this regard, the search for new methods to obtain stimulated macrophages continues.

  For instance, human fibronectin and C-reactive protein have been studied as stimulants of macrophages *in vitro* [67,68] as well as beta-1,3-D- glucan from yeast cell walls [69], lipophilic muramyldipeptide analogs [70], lipoprotein containing lipophilic muramyl tripeptide [71], lipopeptide analog of a fragment from the cell wall of gram-negative bacteria [72].
- **Plant Extracts:** An extract from Crasso-cephalum crepidioides, a plant distributed in Okinawa Islands (Japan), stimulated macrophages to enhance the expression of iNOs and increase the synthesis of NO, and activated antitumor activity against murine Sarcoma 180 (S-180) cancer cells [73].
- **Baicalein** (5,6,7-trihydroxyflavone), isolated from the Chinese herb Scutellaria baicalensis root, can block TGF-β1 via inhibiting PI3K/Akt pathway in M2 macrophages and repolarize them to a M1 phenotype in breast cancer tissues [74].
- An extract from the root of *Panax notoginseng* can re-educate M2-like macrophages toward M1 differentiation [75].
- **Ginsenoside Rb3** from *Panax ginseng* has protective functions against acute lung injury via M1/M2 phenotypic switch [76].
- **Emodin** (1,3,8-trihydroxy-6-methylanthraquinone) bi-directionally regulates both M1 and M2 phenotype programs via different mechanisms including suppression of STAT6 and C/EBPβ signaling to increase H3K27m3 on the M2-related genes. Emodin also restrains excessive M1 or M2 macrophages [77].
- **Osthole** [7-methoxy-8-(3-methyl- 2-butenyl)-2H-1-benzopyran-2-one] is a coumarin member isolated from Cnidiummonnieri (Fructus Cnidii), found to decrease M2-like macrophages in pancreatic tumors by inhibition of STAT6 and p-ERK1/2-C/ EBP β [78].
- **Several "marine" compounds** can regulate macrophages and lead to the polarization of macrophages in both the M1 and M2 phenotypes [79]. Recent studies on the membrane-type di-terpenoids isolated from soft coral species Briareum violaceum in Taiwan demonstrated suppressive effects on iNOS release from the cells, suggesting a potential to shift the M1 phenotype toward the M2 type [80].
- **Polysaccharides** isolated from gorgonian Pseudopterogorgia americana induces the expression of TNF-α, IL-6, and COX-2 in mouse macrophages, but had no effect on the expression of iNOS and NO production.

These compounds decrease expression of proinflammatory cytokines in LPS-activated macrophages, indicating a potential in reprogramming of macrophage polarization toward the M1-type [81].

- **The purine alkaloid homarine**, a major metabolite found in water extracts of Portugal sea anemones, Actinia equine and *Anemonia sulcata*, has a great potential in modulating macrophage polarization [82].

- **Crustaceans of the order Decapoda** (crab, shrimp, prawn, and lobster) are a valuable source of plain polysaccharide and chitosan, which may promote the drug delivery targeting M1 or M2 macrophages [83].
- **Triterpene glycosides** are capable of activating macrophages both *in vivo* and *in vitro* and polarizing them into the M1 phenotype by affecting the purinergic P2X4 receptors pathway [84-86]. Increased ROS and NO levels are found in cells treated with triterpene glycosides.

Car-Engineered Macrophages Act Like M1 Cells (Car-M Cells)

- One of the promising approaches in cell therapy for cancer is the use of CAR initially applied to modify T cells. The CAR-engineered macrophages express pro-inflammatory cytokines and chemokines, convert M2 macrophages to M1, activate the antigen presentation mechanism, recruit and present antigen to T cells, and resist immunosuppressive cytokines. In addition, they exhibit pronounced antigen-specific phagocytosis and *in vitro* tumor suppression.

In two mouse models of solid tumor xenograft, a single infusion of CAR-macrophages reduced the growth of human tumors and increased the overall survival of tumor-bearing animals. In humanized mouse models, it was also shown that genetically modified macrophages induce a pro- inflammatory tumor microenvironment and enhance the antitumor activity of T cells [87]. In a series of recent studies, it was found that the suppression of SIRPα on macrophages from the bone marrow of mice and humans leads to the blocking of recognition of its own "marker of self," CD47, on all other cells.

- **An integrated nanotechnology/biology strategy for cancer immunotherapy** that uses arginine nanoparticles (Arg-NPs) to deliver CRISPR-Cas9 gene editing machinery into cells to generate SIRP-α knockout macrophages and block its binding to CD47 was recently reported [88]. The NP system efficiently co-delivers single guide RNA and Cas9 protein required to knockout the **"don't-eat-me"** signal in RAW 264.7 macrophages. Turning off this signal increased the phagocytosis of human osteosarcoma U2OS cancer cells by fourfold.

Use of Nanoparticles (NPS) As a Carrier for M1 Polarizing Factors

NPs are very tiny particles (<100 nanometers) made up of latex, polymers, ceramic materials, metals, and carbon particles. Their surfaces could be hydrophilic or hydrophobic, and exhibit surface charges and specific ligands, reference for the treatment of various clinical diseases [89]. Other synthetic NPs could have been made using liposomes [90,91], polylactic-co-glycolic acid (PLGA) [92,93], chitosan [94], silica [95], and dextran materials [96]. Applications of NPs in Medical science are increasing due to their physicochemical properties, e.g., size, shape, structure, chemical composition, morphology, and surface properties, etc.

Now-a-days, many researchers are using formulations with small molecules and NPs, such as Toll-like receptor (TLR) agonists, cytokines, antibodies, and RNAs, for macrophage repolarization [97]. Polymeric NPs synthesized with an IL-12 cytokine promote the conversion of M2 to M1-type, and thus could be used for cancer immunotherapy [98]. In 2018, Rodell et al. showed that R848, an agonist of TLR7 and TLR8, shift TAM to an M1 phenotype, and controls the tumor growth [99,100]. Furthermore, since immune cells express mannose receptors, mannose carbohydrate can also be employed to target macrophages [101,102]. Zhao et al. synthesized the albumin NPs having dual ligands, one, a transferrin receptor (TfR)-binding peptide T12, and the second is the mannose. They showed that this therapeutic efficiently can polarize pro-tumor M2 to anti-tumor M1 and inhibit the glioma cell proliferation successfully [103]. Most of mannose are now being applied for macrophage re-polarization as a ligand to target macrophages.

Interestingly, inhibition of CD40 also leads to IL-12 upregulation, which can repolarize TAMs into M1 macrophages. Similarly, inhibition of NF-κB signaling pathway can switch TAMs into M1 macrophages and block tumor cell's growth. Therefore, these approaches have a potential importance in cancer therapy [104]. Another strategy is to make a pH-sensitive NPs. Tumor pH ranges generally from 6.5 to 6.8 while the pH in healthy tissues is 7.4. NPs, like poly(acryl amide) (PAAm), micelles and liposomes can release drugs through protonation or deprotonation designed to be pH-sensitive and bypass the normal tissues [89,105-109].

Theracure Biopolymer Nanovehicles as an Antcancer Regimen

TheraCour platform polymer is a self-assembling, uniform, tailorable linear homopolymer that comprises polyethylene glycol (PEG) within its monomer unit which is heterochemically functionalized with a specially designed linker unit so that covalently connected aliphatic chains are suspended from it and separately site-targeting ligands are also covalently attached to it [110-112] (Figure 4).

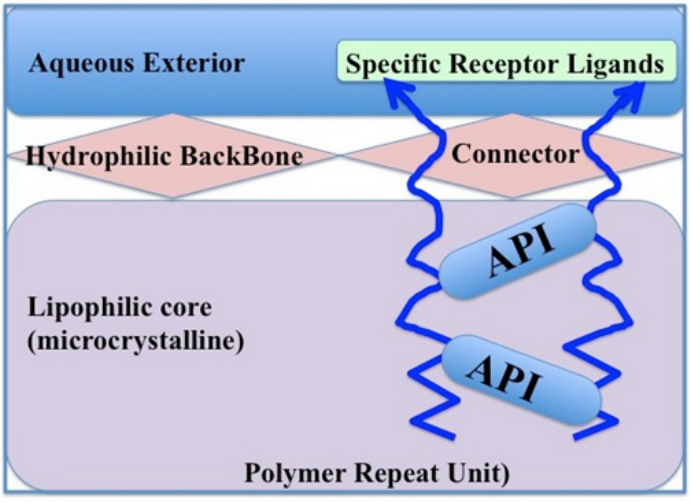


Figure 4: Schematic Presentation of Nanovehicles.

This simple scheme results in a polymer that is like a half-biological membrane, self-assembles into micelles with hydrophobic, flexible core region made of the lipid chains. The hydrophilic ligands directing outwards into the aqueous milieu are ready to seek their partners, connected together by the corona of PEG. Upon binding of the TheraCour polymeric micelle to a cellular receptor may initiate lipid mixing of the flexible pendant interior lipid chains of the micelle with the flexible lipids of the cell membrane, leading to passive fusion. Alternatively, receptor-mediated endocytosis can take place at properly chosen receptors. These processes would result in site-specified or address- targeted delivery of the encapsulated drug payload content of the micelle. The graphical model of anticancer mechansim of TheraCour platform technology are shown in Figure 4. [For Review, 113].

This polymer can effectively encapsulate many types of chemotherapeutic APIs, target the cancer cell based on the selected ligand, and thereby result in effective anticancer activity. Recently it was shown that the cell proliferation of two lung cancer cell lines (A549 and H441), and two breast cancer cell lines (SKBR3 and BT474) are mostly inhibited by a folate-targeted TheraCour polymer delivering API (camptothecin, CPT) [113].

Discussion

Macrophages are the "big eaters" in the animals and humans body capable of engulfing any foreign bodies including bacteria, dead cells, and other particles that are toxic to the body. They also activate T- and B-lymphocytes by antigens presentation.

Macrophages are present in virtually every organ/tissue where they act as the first line of immune defense against pathogens and play an important role in maintaining tissue homeostasis. Classically activated macrophages (M1 phenotype) are stimulated by lipopolysaccharides (LPS) as well as IFN-γ in conjunction with LPS. The "activated macrophage" has an increased ability to kill microorganisms or tumor cells [114].

Polarization toward the M1 direction is accompanied by the secretion of pro- inflammatory mediators. They express receptors for IL-1 (IL-1R1), TLR, and co- stimulatory molecules, the activation of which ensures the amplification of the inflammatory response [115-118]. IL-12 secreted by M1 macrophages also plays a key role in Th1 polarization, whereas IL-1β and IL-23 direct the immune response along the Th17 pathway [115-118].

Alternative activation of macrophages (M2 macrophages) is observed when the cells are stimulated by interleukins, glucocorticoids, immune complexes, TLR agonists, etc. Briefly, M2 phenotype macrophages preferentially activate the motility of cancer cells [119], promote metastasis in stromal and perivascular areas [120], and stimulate angiogenesis in a vascular and peri-necrotic hypoxic areas [121].

In theory, accumulation of macrophages in tumor activate the stroma [122] and ECM remodeling, including lysyl oxidase, MMP9, type IV collagen activation [123]. To make sense of macrophages polarization in cancer remission, studies could be conducted to estimate the number of macrophages sub-type in an outside and inside of the tumor during their different stages of treatment.

Therefore, a complete understanding of nanomaterials interaction with distinct polarized macrophage phenotypes, is important to translate the nanomedicines for clinical purposes. The major points to be considered are:

- Classically activated M1 macrophages are microbicidal and pronflammatory while alternatively activated M2 macrophages are predominantly immune modulators and anti-inflammatory [124].
- The differential uptake methods of nanoparticles by M1 and M2-type macrophages are complex, and involves cytoskeletal remodeling, membrane fusion and vesicular transport [125-127].

Recently it was reported that the polarization of macrophages towards the M1 phenotype resulted an increased uptake ability of non-PEGylated nanoparticles compared to their M2 counterparts [125]. In contrast, inhibition of CD47-SIRPα by anti-CD47 antibodies produced a higher pro-phagocytosis of cancer cells by M1-type as compared to M2-type macrophages [128]. However, how the M1/M2 polarization system works in the tissue-specific macrophages such as microglia in the central nervous system to cause neuro-inflammation remains unknown [129].

In brief, activated macrophage populations possess unique pro- and anti- inflammatory type, which play an important role in immune regulation as well as in disease pathology. During strokes, for example, M1 macrophages becomes active and promote inflammation after ischemic injury, release cytotoxic cytokines and ultimately neuronal death [130]. In contrast, M2-like tumor-associated macrophages promote immune suppression and facilitate tumor invasion [2]. Therefore, the understanding of the mechanism(s) how the nano-materials interact with specific macrophage phenotypes and the ability to design nano-materials for selective targeting to those macrophage subpopulations are crucial parts in designing the nano-medicines. Combining all the above information a conclusive picture therefore can be drawn to combat cancer as follows (Figure 5).

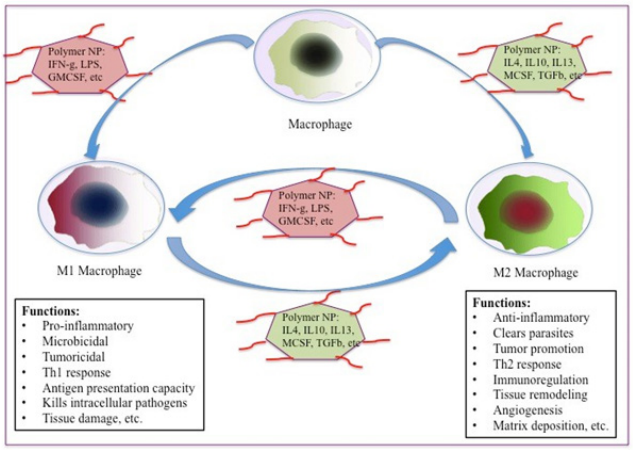


Figure 5: NPs mediated Macrophage Polarization and their Effects.

Conclusion

- In summary, the two functionally distinct macrophage subtypes, M1 and M2, are the key modulators in host immune system against tumors.
- Based on the appropriate system they could act like a "friend or foe," of the tumor cells either to assist or destroy their growth by various mechanisms.
- In direct contact with tumor cells, macrophages attack them with cytotoxic molecules followed by phagocytizing the remnants of the tumor cells.
- In addition, they can activate the production of cytotoxic molecules by cytotoxic T lymphocytes (CTL) and NK cells followed by the production of their specific antibodies by B-lymphocytes.
- However, tumor can "fool" macrophages, and bypass the M1 effect and recruit M2 type for tumor growth.
- In this regard, scientists are trying to find new ways of additional activation of a larger number of macrophages and programming to their M1 phenotype.

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