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Macrophages in multiple myeloma: emerging concepts and therapeutic implications

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Abstract

Multiple myeloma, a clonal plasma cell malignancy, has long provided a prototypic model to study regulatory interactions between malignant cells and their microenvironment. Myeloma-associated macrophages have historically received limited scrutiny but recent work points to central and non-redundant roles in myeloma niche homeostasis. The evidence supports a paradigm of complex, dynamic and often mutable interactions between macrophages and other cellular constituents of the niche. We and others have shown that macrophages support myeloma cell growth, viability and drug resistance through both contact-mediated and non-contact-mediated mechanisms. These tumor-beneficial roles have evolved in opposition to, or in parallel with, intrinsic pro-inflammatory and tumoricidal properties. Thus, simple blockade of protective 'don't eat me' signals on the surface of myeloma cells leads to macrophage-mediated myeloma cell killing. Macrophages also enhance the tumor-supportive role of mesenchymal stem/stromal cells (MSCs) in the niche: importantly, this interaction is bidirectional, producing a distinct state of macrophage polarization that we termed "MSC-educated macrophages". The intriguing pattern of cross-talk between macrophages, MSCs and tumor cells highlights the myeloma niche as a dynamic multicellular structure. Targeted reprogramming of these interactions harbors significant untapped therapeutic potential, particularly in the setting of minimal residual disease, the main obstacle towards a cure.

Multiple myeloma and macrophages: a long-neglected link

Multiple myeloma, a malignant disorder of plasma cells, is the second most common hematological malignancy with approximately 20,000 new diagnoses per year in the United States [1,2]. Its premalignant phase, monoclonal gammopathy of undetermined significance (MGUS), is common in the general population, affecting 4% of Caucasians over the age of 50 [3]. Dramatic changes in the therapeutic landscape in last 10-15 years have prolonged the median survival from 3 years to 6 years or more [4], but the disease remains largely incurable.

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CONFLICTS OF INTEREST
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Myeloma cells are dependent on microenvironmental interactions for their homeostasis under steady-state conditions, as well as to evade stress, such as pharmacological agents administered for therapy [5-7]. We and others have hypothesized that relapse following effective antiproliferative therapy may reflect the persistence of residual tumor cells within tumor-protective, drug-resistant niches in the bone marrow [8-13]. Whether minimal residual disease consists of a distinct tumor cell subpopulation with enhanced self-renewal, and whether this subpopulation is fully committed to the plasma cell lineage, are topics of active investigation and intense debate at present [14,15]. Regardless of the precise identity of the clonal component of minimal residual disease, macrophages are necessary for proper niche orchestration and homeostasis (Figure 1). In this review article, we delineate regulatory interactions between macrophages and other cellular constituents of the myeloma niche and suggest potential therapeutic approaches to redirect these interactions against myeloma tumor cells, particularly in the setting of minimal residual disease [16,17].

Macrophages in hematological malignancies: the more you look, the more you find

Macrophages have emerged as important regulators of cancer-associated inflammation, the seventh hallmark of cancer [18,19]. Although the mechanisms of tumor promotion by tumor-associated macrophages (TAM) have been mostly established from study of solid tumors [20], investigation into the role of tumor-associated macrophages in the evolution of hematological malignancies has recently gained momentum. In lymphoma, increased macrophage infiltration is associated with adverse prognosis, albeit with exceptions. This association appears strongest in the case of Hodgkin's lymphoma [21-23] and more tenuous in non-Hodgkin's lymphomas. Among lymphoma subtypes in the latter category, the presence of large numbers of CD68+ macrophages has been associated with poor prognosis in follicular lymphoma [24,25] but results have been variable in diffuse large cell lymphoma (DLBCL) [26,27]. However, when appropriate markers were used to differentiate between “classically-activated” (or M1-polarized macrophages) and “alternatively-activated” (or M2-polarized macrophages) on DLBCL biopsies, a correlation between macrophage infiltration and adverse outcome was again seen [28] (see below for definition of macrophage polarization states).

In circulating (“liquid”) hematological malignancies, there is some evidence to suggest that macrophages constitute important components of the tumor niche, or site of propagation of clonogenic progenitors. “Proliferation centers” in chronic lymphocytic leukemia (CLL) contain abundant numbers of macrophages and non-macrophage stromal elements [29]. While the significance of the presence of macrophages in these structures needs further study, it is likely that these macrophages also contribute to the survival of clonogenic malignant cells. It is interesting that macrophages in CLL proliferation centers are STAT1-positive, resembling “classically-activated” macrophages. Recent evidence presented at the 2012 American Society of Hematology Meeting suggested that selective depletion of macrophages from an animal model of polycythemia vera could ameliorate clinical manifestations of disease such as spleen size and importantly, the hematocrit, a surrogate of total red cell mass [30]. Therefore, even in “liquid” hematological malignancies, macrophages are likely to have important roles in supporting clonogenic progenitors in the tumor niche, whether located in the bone marrow or peripheral lymphoid organs.

Macrophage polarization in myeloma: nuances in concepts and phenotypes

Macrophages are key components of the myeloid infiltrate of most tumors [20,31]. Tumor-associated macrophages (TAMs) arise from *in situ* maturation of recruited circulating monocytes [32]. Tumors, including myeloma, secrete monocyte-attractant chemokines, such as CCL2 and MIP-1α, abundantly [33,34]. The notion that myeloma-associated macrophages derive from recruited monocytes and not from bone marrow-resident monocytic precursors is further supported by two facts: Firstly, extramedullary plasma cell tumors (plasmacytomas) are rich in tumor-associated macrophages [35]. Secondly, hematopoietic activity in myelomatous bone marrow is suppressed, partly due to the cytostatic effects of cytokines such as TGFβ [36]. Once recruited to the microenvironment of the nascent tumor, monocytes acquire a pro-inflammatory profile ("classically-activated" or "M1-polarized")[37]. In the case of myeloma, macrophage activation within the early lesion may be multifactorial. Myeloma cells secrete inflammatory mediators that promote macrophage activation [38-42]. Mere local tissue disruption from tumoral expansion may elicit or augment innate and adaptive inflammatory responses. Consistently with the notion that the myeloma microenvironment fosters "smoldering" inflammation, myeloma tumor cells express, and respond to signaling from, a broad range of Toll-like receptors [39,43]. Toll-like receptors in the myeloma microenvironment may bind to exogenous ligands carrying pathogen-associated molecular patterns (PAMPs) (components of bacteria and viral pathogens) and/or endogenous ligands carrying danger-associated molecular patterns (DAMPs), such as fibronectin, soluble hyaluronan, heat shock proteins, or endogenous RNA, released in the context of tissue damage, extracellular matrix breakdown, cellular stress, or cell death [43]. Activated oncogenes (particularly RAS homologues) or novel oncogene products (e.g., fusion proteins) may directly elicit potent pro-inflammatory responses [44,45]. Whatever the relevant signals, macrophage activation promotes the synthesis and secretion of pro-inflammatory mediators such as TNFα, IL-1β, IL-6, IL-8 and others [46]. These factors enhance malignant cell growth, protect from stress-inducing stimuli and promote genetic instability and malignant clonal evolution [47]. As tumors expand and progress, selective pressures incite macrophages to acquire characteristics of "alternative activation" or "M2 polarization" [33,48]. M2 macrophages are better suited to carry out tissue–remodeling, to aid local invasion, to promote angiogenesis and to orchestrate a locally immunosuppressive microenvironment.

Although the recognition of macrophage plasticity has been of enormous value in understanding macrophage-mediated modulation of the tumor microenvironment in line with the evolving requirements of the growing tumor, the very concept of "polarization" has led to oversimplification. It is important for M1 and M2 states to be understood as extremes of a dynamic continuum rather than as mutually exclusive cell fates. Importantly, "intermediate" states of macrophage activation may be better suited to the physiology of specific tumor types compared to either of the extremes. Myeloma and activated-type diffuse large B cell lymphoma are prime examples illustrating this principle. Both tumor types are characterized by constitutive NFκB signaling that, in most cases, is non-cell autonomous [49-51]. Macrophages in the immediate tumor microenvironment must be capable of elaborating pro-inflammatory cytokines that elicit constitutive NFκB activity in the tumor cell, particularly when this activity is not conferred by cell-autonomous mutations [52,53]. This "intermediate" state of macrophage polarization may be distinct from "tolerization" [54], because of the continued robust expression of inflammatory mediators [55]. However, the pro-tumoral role of macrophage activation comes at the price of enhanced cytolytic and tumoricidal activity that must be curbed to allow tumor progression (Figure 1). Moreover, distinct states of macrophage polarization may not only be tumor

stage-specific but also tumor site-specific. Macrophages in the expanding invasive rim of the tumor secrete pro-inflammatory cytokines and tissue-remodeling enzymes to allow local invasion as well as recruitment of subsequent waves of inflammatory cells [56]. By contrast, the necrotic center of the tumor is likely to require the presence of M2 macrophages to promote tissue remodeling and angiogenesis [57]. Thus, macrophages at different stages of polarization may co-exist within the same myeloma lesion. Lastly, macrophage polarization is not fixed but may be modulated therapeutically. Several investigators have used therapeutic approaches to reprogram macrophage polarization to elicit strong tumoristatic or tumoricidal effects. Work from the Sondel laboratory has shown that therapeutic activation of macrophages results in effective anti-tumor activity, including in B cell malignancies, that is particularly beneficial in cases where defects in adaptive immunity have arisen as the result of therapy or the tumor pathophysiology *per se* [58-69] (see section on “therapeutic implications”, below). Other investigators have also shown the feasibility and value of this approach in hard-to-treat cancers, such as pancreatic carcinoma [70].

Macrophage activation in the myeloma niche: the central role of TPL2 kinase

In an early landmark study, Brian Durie highlighted the importance of macrophages as a source of paracrine IL-6 in myeloma [71]. Depletion of macrophages from co-cultures with myeloma cells dramatically reduced the growth rate of the latter, an effect simulated by IL-6 depletion. However, it was not clear why macrophages co-cultured with myeloma cells were able to produce excess IL-6. The authors proposed a feedback loop between macrophages and tumor cells in the myeloma niche. According to this model, stimulation of macrophages either by myeloma cells or other components of the microenvironment led to production of excess IL-6 by macrophages which, in turn, promoted myeloma growth and iterative waves of macrophage activation.

These early findings were corroborated and expanded in recent work by our groups [8,55]. We showed that macrophages promoted growth and decreased apoptosis of myeloma cell lines in combined cultures [55]. These tumor-beneficial effects of macrophages were observed in co-cultures with myeloma cells of various genotypes. The growth-promoting effect of macrophages was partially abrogated following treatment with an IL-6-neutralizing antibody. Furthermore, we showed that CD14+ monocytic cells freshly explanted from myeloma bone marrow expressed much higher amounts of IL-6 transcript compared to fresh peripheral blood monocytes from normal donors or macrophages derived from normal peripheral blood monocytes [55]. Mesenchymal stem/stromal cell (MSC)-educated macrophages (MEM, see relevant section below) also expressed high amounts of IL-6 mRNA. Indeed, the inhibitory effect of neutralizing anti-IL-6 antibody was most pronounced in three-way co-cultures of myeloma cells with macrophages and MSCs.

To begin to understand the mechanisms underlying these findings, we focused on TPL2 (Cot, MAP3K8), a serine/threonine kinase with central and non-redundant roles in regulating innate immune responses and cytokine secretion in macrophages [72-75]. TPL2 modulates multiple signal transduction pathways and regulates several pro-inflammatory (TNFα, IL-1β, IL-6, IL-12) and anti-inflammatory cytokines (IL-10, IL-1RA) [73,76-80]. The balance of pro- and anti-inflammatory activities of TPL2 is tissue- and context specific. Our recently published data demonstrated constitutive activation of pro- and anti-inflammatory, TPL2-dependent pathways in myeloma-associated monocytes/macrophages [8]. Furthermore, we established a cell-autonomous effect of TPL2 activity on myeloma cell growth that was attenuated in myeloma cells carrying activating MAPK pathway mutations. Lastly, we observed that TPL2 was activated in myeloma tumor cells undergoing mitosis, raising the possibility that TPL2 activity may be particularly important for regulation of

dividing clonogenic progenitors. Our findings support a model in which TPL2 promotes homeostasis of the myeloma niche by both cell-autonomous and non-autonomous mechanisms (Figure 2). Interference with TPL2 activity may disrupt crucial cross-talk between macrophages and tumor cells in the myeloma niche (see section on “therapeutic implications” below).

Macrophages promote survival of myeloma cells through both contact-mediated and non-contact mediated mechanisms

Work from the Yi laboratory established macrophages as an important component of the myeloma microenvironment by showing that physical interactions between macrophages and tumor cells activate signaling pathways that protect myeloma cells from apoptosis induced by drug treatment [81]. These investigators generated macrophages by treating monocytes with M-CSF followed by treatment with supernatant from cultured myeloma cells to induce the tumor-associated macrophage phenotype. These macrophages were able to prevent drug-induced apoptosis by inhibiting the caspase pathway, especially caspase 3 and PARP. Macrophage-mediated protection from apoptosis was observed with both myeloma cell lines and primary myeloma cells and required direct cell-to-cell contact. Interestingly, IL-6 did not appear to contribute to protection of myeloma cells against drug-induced apoptosis. A subsequent study from the same laboratory established a mechanism behind these observations [82]. They found that the interactions between PSGL-1 and ICAM-1 on myeloma cells and E/P selectins and CD18 on macrophages, respectively, allowed macrophages to protect myeloma cells from drug-induced apoptosis. The interactions stimulated SRC, ERK1/2 kinases and c-MYC and suppressed drug-induced caspase activation.

Contact-mediated support of myeloma cells by macrophages likely acts in concert with non-contact mediated mechanisms. Indeed in our previous study [55], we showed that macrophages protected myeloma cells from apoptosis even when physically separated in transwell plates. Together with findings by the Yi group, our results suggest that macrophages protect myeloma cells from apoptosis through both contact-mediated and non-contact mediated mechanisms. The former may be more important in the setting of drug-induced apoptosis.

Macrophages and osteoclasts belong to the same hematopoietic cell lineage and may support myeloma cell survival and growth, as well as protect myeloma cells from drugs, through common molecular mechanisms (e.g. production of pro-inflammatory cytokines and growth factors [83]). This may suggest that while myeloma cells localized in focal lesions and close to bone surface are protected by osteoclasts, myeloma cells that are localized within the diffuse marrow may be similarly supported by macrophages. Osteoclasts and macrophages may also use common pathways in the induction of angiogenesis in myeloma lesions (see next section) [84].

A bone-resident subpopulation of macrophages has been recently described (osteal macrophages, “OsteoMacs”) [85,86]. Osteal macrophages appear to be involved in bone remodeling and local immunosurveillance. Their role in myeloma niche orchestration and bone pathology merits further investigation and may be significant because they have been shown to be capable to respond to inflammatory stimuli [87] and elaborate pro- and anti-osteoclastogenic cytokines such as TNFα, IL-6, IL-1 and interferon-β. Moreover, OsteoMacs may serve as a pool of osteoclast precursors within myeloma bone lytic lesions.

Macrophages in myeloma angiogenesis and vasculogenesis

Myelomas are highly vascularized tumors and increased vascular density imparts a poor prognosis [88]. Microvascular density in myeloma bone marrows was recently reported to correlate with the prevalence of CD163+ macrophages [89]. Therefore, macrophages are likely to be central orchestrators of the “angiogenic switch” in myeloma, similar to other tumors [90]. Macrophages are likely to promote neoangiogenesis through both cytokine secretion and physical contribution to the generation of a vascular network.

A major mechanism behind angiogenic induction by macrophages is through secretion of vascular endothelial growth factor- A (VEGF-A) in poorly vascularized areas of tumors [91]. A paracrine loop between myeloma cells and stroma ensures robust induction of angiogenesis as myeloma lesions expand [92]: macrophages secrete VEGFs to promote myeloma cell growth and angiogenesis, leading to further waves of myeloma cell-derived secretion of VEGF-A and basic fibroblast growth factor (bFGF) that directly contributes to angiogenesis but also induces stromal cells to secrete VEGF-C and –D that stimulate myeloma cell growth through VEGFR-3 in a self-perpetuating loop [93]. Additionally, activated macrophages synthesize nitric oxide, leading to vasodilation and enhanced angiogenesis [94]. Lastly, the angiogenic factors secreted by macrophages stimulate mast cell migration [95,96], and mast cells contribute to angiogenesis [97,98].

Intriguing observations from the Ribatti and Vacca groups have highlighted direct, structural contributions of macrophages to the myeloma blood vessel network by “vasculogenic mimicry” [99]. In a study by Scavelli *et al.* [100], they reported that bone marrow macrophages from myeloma patients assumed a vascular endothelial cell-like phenotype when activated with VEGF and basic fibroblast growth factor (bFGF). In contrast, macrophages from healthy donors, non-active myeloma and monoclonal gammopathy of unknown significance (MGUS) did not exhibit similar behavior. Importantly, the angiogenic and vasculogenic properties of bone marrow macrophages in myeloma were inhibited following treatment with the proteasome inhibitor, bortezomib, as well the bisphosphonate, zolendronic acid [101].

Macrophages in the myeloma niche: friend or foe?

The literature presented above supports the hypothesis that macrophages are integral components of the myeloma niche and support myeloma cell growth and viability under both steady-state and stress conditions as well as promote niche angiogenesis and vasculogenesis. However, recent work from the Weissman lab has shown that macrophages also possess inherent tumoricidal potential that would be detrimental for malignant plasma cells if the latter did not express protective “don't eat me” signals [102]. Indeed, myeloma cells, both primary cells and lines established in culture, universally upregulate CD47, an integrin-associated receptor protein. CD47 interacts with SIRPa on the surface of myeloma-associated macrophages to deliver a potent anti-phagocytosis (“don't eat me”) signal [103]. Simple inhibition of this interaction by a CD47-blocking antibody elicits frank tumoricidal responses by macrophages, resulting in tumor regression in xenotransplantation models, including models utilizing human primary myeloma cell grafts into human fetal bone implants [102]. Importantly, anti-CD47 antibody bound on myeloma cells did not induce complement-mediated lysis or antibody-dependent cell-mediated cytotoxicity (ADCC). These results demonstrate that macrophages in the myeloma niche display inherent anti-tumor potential and simple perturbation of the balance between macrophage activation and defenses put up by myeloma cells suffices to elicit macrophage-mediated tumor regression.

MSC-educated macrophages: a novel subtype of the alternatively-activated macrophage?

Bone marrow mesenchymal stem/stromal cells (MSCs) are thought to play major regulatory roles in the myeloma microenvironment [5,7,13,104]. Myeloma cells receive key supportive signals from MSCs [105-107], including MSC-derived cytokines that are important for growth and survival of myeloma cells [108-111]. Moreover, cell adhesion is thought to be another mechanism by which bone marrow MSCs support myeloma cell survival [112,113].

Macrophages induce MSCs to express IL-6, CCL5, and interferon gamma-induced protein-10 (CXCL10) and to exhibit increased mobility in response to multiple soluble factors produced by macrophages including IL-8, CCL2, and CCL5 [114]. Macrophage-MSC cross-talk is bidirectional: the interaction results in a distinct state of macrophage polarization that we have termed “MSC-activated macrophages” (MEM) [115]. MEMs bear many phenotypic characteristics of M2 polarization, such as expression of the surface marker CD206. However, the pattern of cytokine secretion by MEMs is unique: high IL-10, low IL-12, low TNFα and high IL-6. Whereas high IL-10 and low IL-12 levels are characteristic of the “alternatively-activated” M2 phenotype, the continued expression of high levels of IL-6 sets MEMs apart from classical M2 macrophages, although there is some recent evidence to suggest that IL-6 may have a role in M2 polarization [116]. With regard to TNFα, it should be noted that low-level tonic stimulation of myeloma cells may be more optimal for tumor propagation compared to acute or steep surges in availability of this pleiotropic cytokine in the tumor microenvironment [117]. Moreover, TNFα signaling may become pro-tumoral in the presence of *RAS* mutations, a frequent genetic alteration in myeloma [118-120]. This MEM-specific cytokine profile was corroborated by the findings of Zhang *et al.*, who showed that MSC-conditioned medium induced higher expression of IL-6 and IL-10 but lower level of TNFα in macrophages [121]. MSCs also modulated cytokine release by macrophages in a study by Maggini *et al.* [122]. The pattern of cytokine expression characteristic of MEMs renders this cell type particularly suitable for the support of tumor cells in the myeloma niche.

MSCs modulate macrophage polarization in the niche but also they orchestrate monocyte recruitment to sites of active tumor cell propagation. Thus, in follicular lymphoma, MSCs in the niche upregulate CCL2 to recruit inflammatory monocytes [123]. Follicular lymphoma-derived MSCs cooperate with macrophages to sustain malignant B cell growth, at the same time as skewing macrophage polarization towards a pro-angiogenic and LPS-unresponsive phenotype [123]. Similar results were recently confirmed in mouse models of lymphoma [124,125]: interestingly, the ability of lymphoma-derived MSCs to promote lymphoma growth was abolished in CCR2-null mice, demonstrating that MSC support of tumor cells required the recruitment and presence of monocytes/macrophages. Control marrow-derived MSCs acquired the tumor-promoting properties of lymphoma-derived MSCs when pre-treated with TNFα [124]. Thus, bidirectional crosstalk between MSCs and macrophages orchestrates a tumor-protective niche.

Macrophages in myeloma therapy: repolarization versus depletion

The last decade has witnessed the advent of several new therapies for multiple myeloma [2,126]. In addition to cytotoxic chemotherapy, including high-dose chemotherapy followed by autologous stem cell rescue (autologous transplant), proteasome inhibitors [127] and thalidomide analogues (thalidomide, lenalidomide) are used [128,129]. These approaches have prolonged survival for many myeloma patients. Myeloma therapies mainly act by direct antiproliferative effect on tumor cells. “Immunomodulatory” activities have been ascribed to thalidomide analogues based on *in vitro* observations [128], however the

significance of the immunomodulatory effect ***in vivo*** is unclear, particularly as they are often co-administered with potent immunosuppressive agents, primarily steroids [130,131]. It is important to note however, that an inhibitory effect of thalidomide and its analogues on TNFα production by LPS-stimulated monocytes was recognized early [132,133]. Compared with thalidomide, inhibition of TNF⟨ was 2000-fold more potent with lenalidomide and 20000-fold more potent with pomalidomide [134]. These observations suggest that thalidomide analogues may directly modulate the activation/polarization status of myeloma-associated monocytes/macrophages.

Despite the success of traditional and novel agents, myeloma remains virtually incurable. Current therapies, including autologous transplantation, cannot eradicate the disease in most patients and even after allogeneic transplantation, relapses are frequently seen [135]. We hypothesize that the persistence of residual tumor cells nested within tumor-protective niches constitutes a major mechanism for relapse and that macrophage-tumor cell interactions are crucial determinants of the homeostasis of the myeloma niche. Therefore, targeted approaches to redirect these interactions may overcome the limits of current therapies and potentially lead to a cure.

Four possible approaches to therapeutically exploit macrophage activation and function in the myeloma niche can be envisaged. First, reprogramming of macrophages to an overtly activated phenotype through M1-polarizing signals (eg. CD40-agonistic antibody). Second, interference with signaling pathways that promote shift to an “alternatively activated” M2 phenotype or activate anti-inflammatory responses, e.g., through IL-10 inhibition or TPL2 blockade. Third, inhibition of “don't eat me signals” on myeloma tumor cells (anti-CD47 antibody). Fourth, interference with monocyte recruitment to the niche (eg. through CCL2-CCR2 axis inhibition) or selective depletion of tumor-associated macrophages. Combinations of any of these approaches might have additive or synergistic effects.

Therapeutic repolarization of macrophages to an “unopposed” M1 phenotype has been achieved following the administration of signals that directly and potently activate intracellular pro-inflammatory pathways. Pioneering work from the Sondel group has demonstrated that it is possible to repolarize macrophages through administration of a first, or “priming” signal (CD40 ligation through an agonistic CD40 antibody) followed by a second, or “triggering” signal that has typically consisted of a Toll-like receptor (TLR) ligand [58-62,64,66-69]. Although *in vitro* assays have utilized lipopolysaccharide (LPS) for TLR stimulation, concerns about the systemic toxicity of endotoxin-type agents have led to exploration of CpG, a TLR9 ligand, as “triggering” signal. Indeed, the combination of CD40 ligation and CpG-mediated macrophage activation is cytostatic and cytocidal *in vitro* and leads to tumor regression *in vivo*, through elaboration of factors such as NO, TNFα and TRAIL [58]. More recently, the Vonderheide group has shown that administration of an agonistic CD40 antibody together with chemotherapy led to meaningful clinical responses in a particularly recalcitrant tumor, pancreatic carcinoma [70]. Although initially the investigators hypothesized that the effect was lymphocyte-driven, dissection of the relevant mechanisms in a genetically-engineered animal model demonstrated that the anti-tumor effects were mediated entirely by activated macrophages. In B cell malignancies and other cases where the malignant cells express CD40, there is a potential concern that CD40 stimulation may lead to (at least transient) tumor stimulation and growth. However, in a model of CLL (a CD40-expressing malignancy), a modest effect on tumor cell proliferation was overcome by macrophage-mediated tumoricidal activity following anti-CD40 antibody administration [62]. Moreover, binding of CD40 antibody on the surface of CD40+ tumor cells may kindle a primary tumoricidal effect through antibody-mediated cell cytotoxicity (ADCC), acting in parallel with macrophage activation [58].

Direct interference with immunosuppressive pathways may offer an alternative or complementary approach to reprogram macrophages to an anti-tumor role. Disruption of IL-10-dependent immunosuppressive pathways have been explored in cancer immunotherapy [136]. Targeted inhibition of the IL-10 axis may be achieved through small molecules targeting TPL2 kinase activity. Indeed, in a genetically-engineered model of colonic carcinogenesis, genetic ablation of *Tpl2* led to enhanced inflammation and tumor promotion, predominantly through inhibition of IL-10 production coupled with defects in regulatory T cell (Treg) generation [137]. Pharmacological TPL2 inhibitors have been under continuous development for over a decade [138-144]. TPL2 has low homology to other kinases and unique structural features in its ATP-binding loop that will likely allow the design of highly specific inhibitors [75]. Moreover, Tpl2 activity is not inhibited by staurosporine, a non-specific kinase inhibitor [145]. However, the lack of crystal structure has hampered the pace of TPL2 inhibitor development. Several classes of compounds have been shown to have good activity in kinase inhibition assays as well as *in vivo* activity, by blocking TNFα responses to systemic LPS administration [146]. A natural compound, luteolin, has recently been shown to inhibit TPL2 activity, albeit with a high IC_{50} [147]. Because *Tpl2* nullizygosity is compatible with normal hematopoietic development and function [148], therapeutic TPL2 blockade is likely to be well tolerated in patients with hematological malignancies.

A third approach involves blocking of “don't eat me’ signals on the surface of tumor cells. Anti-CD47 antibody-based approaches in particular have found applicability in several tumor models and are fast moving to the clinic [103]. It is unlikely that anti-CD47-based approaches will suffice as monotherapy, particularly in the setting of bulky disease. However, CD47 blockade may be particularly attractive in the setting of minimal residual disease, alone or in combination with antiproliferative therapies. Interestingly, cancer stem cells appear to upregulate CD47 strongly, making anti-CD47 therapy particularly attractive in those tumors where minimal residual disease may have stem-cell characteristics [149].

Lastly, therapeutic interference with monocyte recruitment or selective depletion of tumor-infiltrating macrophages may delay tumor growth, e.g. through inhibition of angiogenesis, as well as disrupt essential cross-talk with other constituents of the niche. Monocytes are recruited to nascent tumors through their expression of CCR2, the receptor for the potent monocyte chemo-attractant cytokine CCL2, produced by the tumor microenvironment [150]. Indeed, inflammatory monocytes in both mice and humans are CCR2+ and monocyte migration through the CCR2-CCL2 axis is important for the generation of the metastatic niche [151]. There are several CCR2 inhibitors in development (reviewed in [152]). Although the main applications have so far focused on therapy of autoimmunity, atherosclerosis and metabolic disease, CCR2-CCL2 axis inhibition is beginning to be explored in the context of cancer. Clinical trials using anti-CCL2 antibodies, alone or in combination with chemotherapy, have been conducted in solid tumors aiming at inhibition of angiogenesis (www.clinicaltrials.gov). Robust expression of CCL2 by the rich vascular network of myeloma bone marrow [153,154] underscores CCL2-CCR2 axis inhibition as a particularly attractive therapeutic strategy in myeloma. Lastly, selective depletion of tumor-infiltrating macrophages may be envisaged. Powerful proof-of-principle in favor of therapeutic macrophage targeting was recently provided by the demonstration that trabectedin, a novel marine-derived compound, exerted powerful anti-tumor effects through depletion of monocytes/macrophages and associated collapse of tumor vascular networks [155]. Trabectedin, or similar approaches, may hold great promise in macrophage-rich, vascular tumors, such as myeloma.

Conclusions

Recent and expanding investigations suggest that macrophages play a major, and hitherto poorly appreciated, role in the development and propagation of hematological malignancies, including multiple myeloma. Work from our laboratories and others, has provided insight into the bidirectional interactions between macrophages and malignant plasma cells and between macrophages and MSCs in the myeloma microenvironment. Taken together, these mechanistic studies support the hypothesis that macrophages are central to the homeostasis of the myeloma niche and therefore, therapeutic approaches that exploit these interactions may hold the key for improved control and ultimately cure in patients with myeloma.

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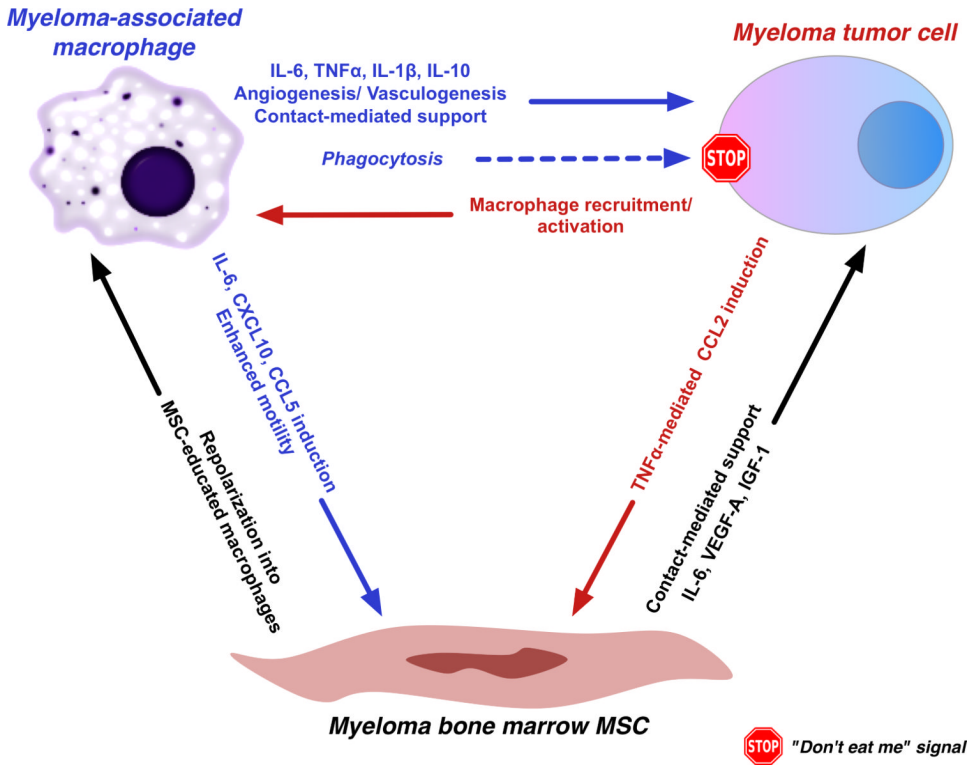

Figure 1. Regulatory interactions between macrophages, mesenchymal stem/stromal cells (MSCs) and malignant plasma cells in the myeloma niche

Macrophages directly support malignant plasma cells through contact-mediated interactions, cytokine secretion and indirectly, through orchestration of the “angiogenic switch” and an immunosuppressive environment conducive for tumor cell propagation. These tumor-beneficial roles are balanced by inherent tumoricidal and phagocytic properties of activated macrophages. Myeloma-associated macrophages also engage in bidirectional interactions with mesenchymal stem/stromal cells (MSCs) and the latter, in turn, modulate the polarization state of macrophages (“MSC-educated macrophages”, see text) as well as provide direct support to tumor cells.

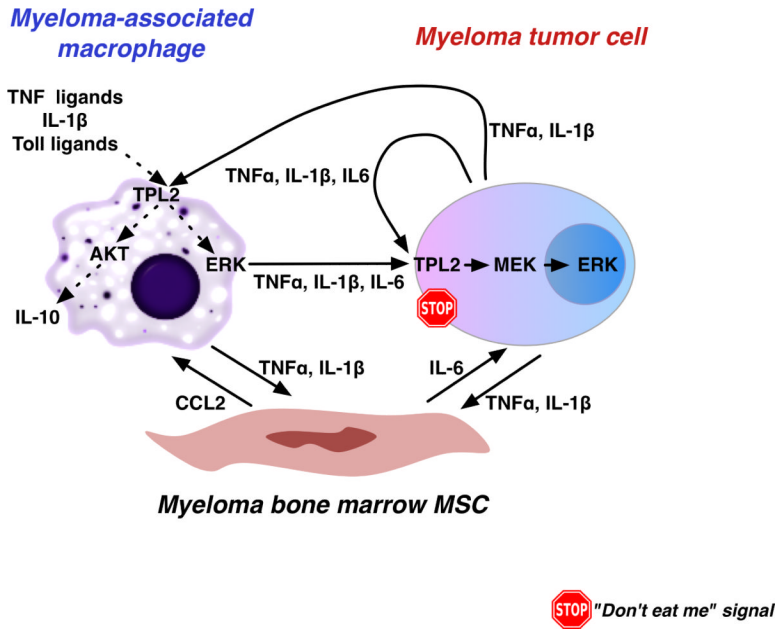

Figure 2. TPL2 kinase regulates myeloma growth through tumor cell-autonomous and non-autonomous mechanisms, the latter involving myeloma-associated macrophages

TPL2 is a key regulator of cytokine secretion by myeloma-associated macrophages. In malignant plasma cells, TPL2 activates downstream MAP kinases in response to growth and inflammatory signals. Targeted inhibition of TPL2 activity may disrupt crucial regulatory cross-talk between macrophages and malignant cells in the myeloma niche.