

The significant role of mast cells in cancer

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Abstract Mast cells (MC) are a bone marrow-derived, long-lived, heterogeneous cellular population that function both as positive and negative regulators of immune responses. They are arguably the most productive chemical factory in the body and influence other cells through both soluble mediators and cell-to-cell interaction. MC are commonly seen in various tumors and have been attributed alternatively with tumor rejection or tumor promotion.

Tumor-infiltrating MC are derived both from sentinel and recruited progenitor cells. MC can directly influence tumor cell proliferation and invasion but also help tumors indirectly by organizing its microenvironment and modulating immune responses to tumor cells. Best known for orchestrating inflammation and angiogenesis, the role of MC in shaping adaptive immune responses has become a focus of recent investigations. MC

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mobilize T cells and antigen-presenting dendritic cells. They function as intermediaries in regulatory T cells (Treg)-induced tolerance but can also modify or reverse Treg-suppressive properties. The central role of MC in the control of innate and adaptive immunity endows them with the ability to tune the nature of host responses to cancer and ultimately influence the outcome of disease and fate of the cancer patient.

Keywords Mast cell · Mast cell progenitor · Tryptase · Chymase · Cancer · Tumor · Colon-cancer · Polyposis · Regulatory T Cell · Immune Surveillance

1 Mast cell subsets and tissue distribution

MC are tissue-resident sentinel cells. MC progenitors (MCP) have been suggested to branch off very early from hematopoietic stem cells [1] or alternatively to differentiate late in the myeloid lineage from the granulocyte monocyte progenitor and have a common precursor with basophils [2]. At least two distinct subpopulations of rodent MC have been identified based on morphologic characteristics, tissue localization, and protease content [3–6]. Mucosal MC can be distinguished from connective tissue MC by expression of chymase instead of tryptase and for lower expression of heparin in the secretory granules. Human MC are also divided into two types depending on the expression of tryptase, chymase, and other proteases in their granules [7]. MC, which contain only tryptase, are referred to as MC_T and typically colocalize with T cells in the respiratory and intestinal mucosa. MC that contain tryptase, chymase, and other proteases, such as carboxypeptidase A and cathepsin G, are referred to as MC_{TC} and are found in connective tissues, including skin, submucosa of the gastrointestinal tract, breast parenchyma, myocardium, lymph nodes, conjunctiva, and synovium.

In mice, mature MC are rarely present outside the connective tissues. In the intestine, isolated MC are detected in the mid-crypt region along with epithelial stem cells. Affinity of mature MC to stem cells is also highlighted by their localization in the vicinity of hair follicle stem cells and their involvement in regulating the transformation from resting (telogen) follicle to active hair growth (anagen) [8]. Other than these exceptions, MC normally migrate and reside in tissue as progenitors [9]. Both the intestine mucosa and hair follicles are rich sources of MCP [10, 11]. Committed but undifferentiated MCP reside within the lymph hematopoietic system comprising the bone marrow, spleen, peripheral blood, mesenteric lymph node, and gut mucosa. These progenitors differentiate into chymase-expressing mature MC upon challenge [1, 12, 13]. Primary MC expanded *ex vivo* have characteristics of both connective and mucosal MC irrespective of the tissue source.

Extensive work has demonstrated plasticity of MC subsets in tissues [14, 15], showing that subtype classifications are not rigid and may be shifting within the tumor microenvironment. For example, MC that mediate immunosuppression in mice in tolerant allografts have been suggested to be distinct from other MC [16]. Different subsets of MC infiltrate tumors at different stages of tumor progression. In this respect, we have detected strictly intraepithelial chymase-expressing MC in mouse benign adenomatous polyps [10], while MC infiltrating invasive carcinomas in mice are predominantly found in the invasive borders of tumors, as well as in tumor stroma, the muscularis mucosa and submucosa, and typically express tryptase [17] (Fig. 1).

2 Recruitment of mast cell progenitors

MC exit the bone marrow as committed progenitors before trafficking through the circulation to their target tissues,

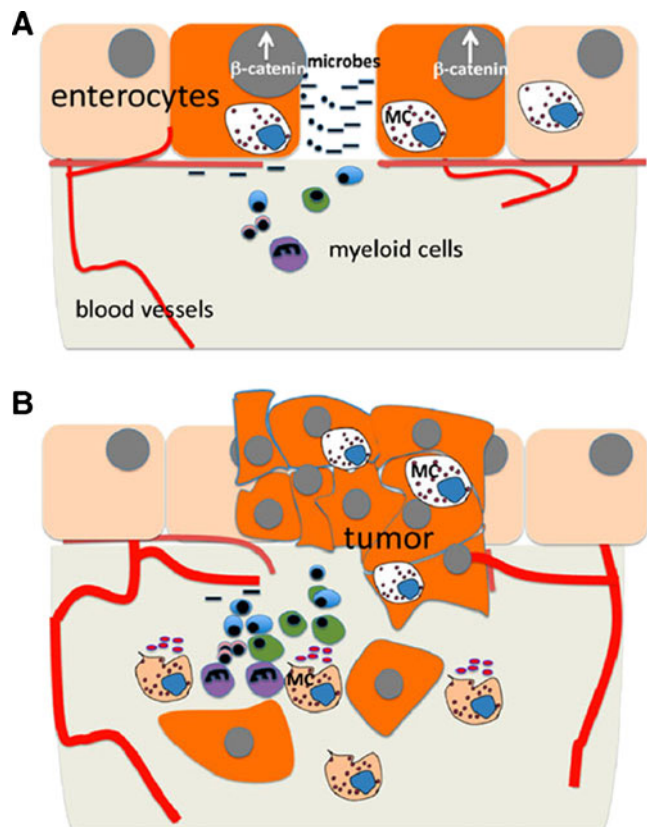


Fig. 1 Different subsets of MC infiltrate tumors at different stages of tumor progression. **a** Following stabilization of β -catenin, benign dysplastic lesions are decorated with intraepithelial MC (white cells). MC contribute to tissue reorganization that breakdowns epithelial barriers and allows access of microflora to the mucosa. MC also promote angiogenesis and recruit secondary pro-inflammatory cells. **b** Tumor invasion is facilitated by stromal and submucosal MC (pink cells) that appear at the invasive border of the tumor and in the tumor stroma. These are typically degranulating

where they remain as sentinel cells and ultimately respond to challenge by undergoing terminal differentiation [15, 18, 19]. Highest densities of MCP are found in the skin, airways, and digestive tract, where they are thought to act as a first line of defense against infiltrating pathogens and parasites [20]. As sentinel cells, MC are first in the line of innate immune cells responding to tumor initiation, as shown with inducible mouse models of polyposis [10]. The mechanisms governing tissue-specific MCP recruitment and differentiation vary with target tissue and stimulus within the tissue [21, 22]. In contrast to many other myeloid subpopulations, MC are capable of proliferating after full maturation. Thus, focal mastocytosis as seen in tumors is likely to reflect activation of sentinel MCP, recruitment of MCP from bone marrow, local proliferation, differentiation, as well as proliferation of mature MC.

Human cord blood-derived MCP predominantly use $\alpha 4$ -integrin, VCAM-1, PSGL-1, and other ligands that bind E-selectin for adhesion to cytokine-activated human endothelial cell monolayers. This explains the abundance of MC at sites of mucosal inflammation, where VCAM-1 and E-selectin are important inducible receptors [23]. In mice, homing of MCP to the intestine or lungs depends on autologous expression of CD49d $\beta 7$ ($\alpha 4\beta 7$ integrin) and VCAM-1 as well as the chemokine receptor CXCR2 [24–27]. It is not known to what extent recruitment of MCP to tumors and their intratumor proliferation is dependent on these factors. Among potential candidates for recruiting MCP to tumors, stem cell factor (SCF) has attracted much attention.

MCP and MC express high levels of c-KIT (CD117) and are strongly SCF-dependent [28]. SCF is critical for MC survival, differentiation, proliferation [29], migration [30], and function [31–33]. Besides MC, expression of c-KIT is normally restricted to germ cells, melanocytes, and intestinal Cajal cells [6, 34, 35] but is also up-regulated in tumor cells [36]. Autocrine/paracrine stimulation of c-KIT has been observed in colorectal carcinoma cell lines, and approximately 10% of patients expressed c-KIT together with SCF in their adenoma or primary tumors [37]. Not surprisingly, tumor produced SCF attracts MCP [38]. SCF-induced MC migration is dependent on PI3-K p85 [39] and activation of the Fyn kinase downstream of c-KIT [40].

MCP recruitment and tissue density are regulated by T cells. Mice with polyposis have increased density of MCP in the intestine, and adoptive transfer of Treg from healthy mice to polyp-ridden mice causes a significant drop in their frequency [41]. Paradoxically, Treg orchestrate MC recruitment to inflamed lung in mouse models of antigen-induced bronchial asthma [42]. This may be of significance in tumors and tumor-draining lymph nodes, where Treg have preferential access and accumulate [43–48]. There are other examples where MCP recruitment is T cell-dependent, such as the response to the gut parasite *Trichinella spiralis*, which is

hampered in congenitally athymic mice [49]. MC recruitment can be in response to soluble factors produced by T cells [50]. In addition, dendritic cells [51] are implicated in recruiting MCP to the mucosal tissue in mice. In contrast to many other myeloid subpopulations, MC are capable of proliferating after full maturation. Thus, focal mastocytosis, as seen in tumors, is likely to reflect activation of sentinel MCP, recruitment of MCP from bone marrow, local proliferation, differentiation, as well as proliferation of mature MC.

T cell dependence of MC recruitment is evident from patients with defective T lymphocyte function. These patients lack tryptase-positive chymase-negative MC in gastrointestinal mucosa [52]. In mice, Treg control antigen-induced recruitment of MCP to the lungs [53]. However, MCP can also be recruited to the mucosal tissue in the absence of any T cells, since immune-deficient mice have a normal content of MCP in the spleen and intestine [10]. If T cells are involved in recruiting MC to tumors, then they must be dispensable, since we have observed unperturbed if not enhanced focal mastocytosis in adenomatous polyps that emerge in immune-deficient Rag1^{-/-} Min mice [10]. Similarly, lymphocyte independent infiltration of MC has been reported in the synovia of mice prone to rheumatoid arthritis [54].

3 Mast cell arsenal of effector functions

MC produce three categories of effector molecules. One category includes those effector molecules which are stored in granules such as serotonin, histamine, heparin, tryptase, and chymase. Another includes those that are synthesized *de novo* upon cell stimulation such as lipid mediators (PAF), prostaglandins (PDG2), and leukotrienes (LTB4, LTD4). Lastly, a large variety of cytokines that are Th1-associated (IFN- γ , IL-2, IL-3, GM-CSF, and TNF- α), Th2-associated (IL-4, IL-5, IL-6, IL-10, IL-13, IL-33, and GM-CSF), or TH17-biased (TGF- β , IL-6, IL-1 β , and TNF- α), chemokines and angiogenic factors including vascular endothelial growth factor [55] and tryptase [56] as well as proteases including tryptases, chymases, cathepsins, and carboxypeptidase. Release of mediators by MC occurs either within minutes of activation (immediate acute) or over hours (delayed) depending on whether these are pre-made and stored in granules for immediate release or require *de novo* synthesis.

IL-1 β , IL6, and TNF- α released by MC are pro-inflammatory cytokines that can generate TH17 cells, inactivate Treg, or otherwise render them pro-inflammatory. MC are a source of preformed TNF- α [57], which also works as an autocrine MC stimulatory factor, and its elimination negatively impacts MC density in the gut [10]. MC release TNF- α after incubation with bacteria, providing a potent chemotactic factor for neutrophils [58–60]. MC deficiency has consequences for control of microbes. The MC-deficient

W-sh mice have chronic intestinal inflammation while TNF- α -deficient mice have increased mortality in the “cecal ligation and puncture” model compared with wild-type mice [61]. Other MC-derived products that contribute to the influx of neutrophils and control of microbes, include leukotriene B4 (LTB4), human tryptase β I, macrophage inflammatory protein (MIP)-1 α (CCL3), MIP-1 β , MIP-2, monocyte chemoattractant protein-1, RANTES (CCL5), and IL-8 (CXCL8) [62].

MC can produce large quantities of IL-1 β [63] that may be processed by MC chymase or potentially by caspase-1 in an Nlrp3 inflammasome-dependent manner. It is also known that MC can induce production of IL-1 β by macrophages, at least in the pathogenesis of rheumatoid arthritis [64]. IL-1 β has a key role in chronic inflammatory reactions that help tumors flourish [65, 66]. IL-1 β is also an important angiogenesis mediator regulating the synthesis of angiogenesis factors. A previous study has shown that human MC produce angiogenesis factor IL-8, when stimulated with IL-1 β [67]. It is tempting to speculate a connection between NLRP3 inflammasome activation in MC and cancer since NLRP3 and IL-1 β are associated with environmental silica and asbestos carcinogenesis [68].

Of the long list of cytokines that can be released by MC, IL-10, and TGF- β deserve particular attention due to their role as immune-suppressive mediators that also generate induced Treg (iTreg). Secretion of IL-10 by MC has been implicated in down-regulation of antigen-specific immune responses by mosquito bites [69]. However, MC also respond to IL-10 differentially depending on the cellular source and level of activity. IL-10 can inhibit MC degranulation by suppressing MC IgE receptor expression and signaling [70] while blocking antibodies to IL-10 can hinder antigen-induced recruitment of MCP to the lungs of C57BL/6 mice [53].

In the mouse, at least five different chymases (mMCP-1, mMCP-2, MMCP-3, MMCP-4, and MMCP-5) and three different granule-associated tryptases (mMCP-6, mMCP-7, mMMP-11/transmembrane tryptase) have been described [71]. C57BL/6 mice are defective in mMCP7 leaving them with mMCP6 as the major tryptase [72]. Expression of proteases in mouse MC is strictly related to the type of MC. Thus, mucosal MC express mMCP-1 and mMCP-2, whereas connective tissues MC express mMCP-3, mMCP-4 mMCP-5, mMCP-6, mMCP-7, and carboxypeptidase [73, 74].

MC have significant cyclooxygenase and lipoxygenase activity and generate inflammatory lipid metabolites of arachidonic acid. In mice, the major cyclooxygenase product of MC are prostaglandin-D2 (PGD2) and prostaglandin E2, and the major lipoxygenase products are LTC4, LTD4, and LTE3 [75]. LTB4 a MC product of 5-lipoxygenase is a chemoattractant for MC progenitors [76] and was recently shown to be a potent polypypsis

promoting factor in mice [77]. Human MC also produce LTB4, although in much smaller quantities than PGD2 or LCT4. MC are a source of platelet activating factor, and platelets are known to augment the growth and dissemination of primary tumors by promoting angiogenesis, immune evasion, and tumor extravasation [78].

Release of certain mediators by MC requires degranulation. Degranulation is responsible for release of proteases as well as powerful anticoagulants such as heparin, chymase, and tryptase. Mechanisms of MC degranulation are best described in the context of the development of immediate hypersensitivity. The cross-linking of Fc ϵ RI, the high affinity IgE receptor, by allergen- or tumor-specific immunoglobulin IgE on MC is the primary trigger for the rapid release of their granules by exocytosis [79]. PI3K plays a key role in MC biology including degranulation [80]. MC treated with LY294002 (PI3K inhibitor) or inhibition of PI3K by over-expression of the dominant negative inhibitor Δ p85 leads to a significant decline in MC degranulation via antigen-induced Ca²⁺ signals [81].

MC may also be activated by “alternative,” IgE-independent pathways, such as aggregation of low-affinity Fc γ RIII IgG receptors by IgG/antigen complexes, c-Kit, and pattern recognition Toll-like receptor mechanisms, activation of the complement receptors (C3aR, C5aR, CR2, CR4) by exposure to chemokines, anaphylatoxins C3a and C5a, fragments of fibrinogen, and fibronectin [76, 82–87]. A recent study suggests that the release of micro-particles from activated T cells induces MC degranulation and release of cytokines via the MAPK pathway independent of IgE [88]. These alternative pathways are thought to work through vesicle-mediated degranulation which involves small aliquots of granule-associated material that detach from the granule membrane for selective paracrine or endocrine transport to the cell exterior (reviewed by [89, 90]). This degranulation pattern has frequently been observed in MC infiltrating areas of chronic inflammation or tumors [91]. The alternative mechanism appears to be responsible for MC release of tumor-promoting cytokines and lipid mediators, particularly in early stages of cancer initiation such as in benign adenomatous polyps where degranulation MC is not a major event [10].

4 Human studies showing correlation between mast cells and cancer progression

A number of studies have documented correlations between the presence of MC and tumor development [92–98]. MC infiltration in tumor is an independent prognostic factor and predictor of poor outcome in prostate cancer [99] and has been heralded as a novel prognostic marker [100]. Expression of c-Kit has been shown to predict recurrent

disease and is suggested to be a marker of fibroepithelial phyllodes tumors of the breast [101], but a recent report attributes this expression to the presence of infiltrating MC [102]. High MC score is associated with unfavorable prognosis in patients with follicular lymphoma treated with immunochemotherapy [103]. Increased MC counts, tumor size, and lymphovascular invasion are associated with an adverse prognosis in Merkel cell carcinomas [104]. MC infiltration in Hodgkin lymphoma also demonstrated a poor prognosis associated with infiltration of CD30L-expressing MC [105]. Intriguingly, this effect appears to occur independent of MC-mediated effects on angiogenesis [106], potentially via direct interaction between MC and Hodgkin and Reed–Sternberg cells expressing CD30. MC are etiologically associated with the formation of neurofibromas in human neurofibromatosis1 patients [107]. Tumor promotion by MC is attributed to the release of mediators of angiogenesis and recruitment of macrophages, neutrophils, and eosinophils (for reviews see [17, 108]).

High MC density together with angiogenesis was predictive of poor clinical outcome in colorectal cancer [109–111], lung cancer [95], and pancreatic cancer [112]. Positive correlation between MC and microvessel densities was observed in colorectal cancer [109–111] and lung cancer [113, 114], supporting the involvement of MC in the tumor angiogenic process. MC tryptase can be detected in the peripheral blood of pancreatic cancer patients, presumably reflecting the abundance of tumor-infiltrating MC [112]. In hepatocellular carcinoma, higher peritumoral MC density was associated with worse clinical outcomes and shorter recurrence free survival, while higher density of MC was related to increased probability of early recurrence. Interestingly, peritumoral Treg were positively correlated with MC density and reversely related to clinical outcomes [115, 116].

5 Tumor promotion by mast cells in mouse models of cancer

Essential roles of MC in tumor progression are documented in a number of mouse models of cancer. The angiogenic potential of MC in cancer was highlighted by a report from the laboratory of Doug Hannah where development of blood vessels in skin tumors that developed in a transgenic mouse model of human papilloma virus-16 was shown to be MC-dependent; notably, premalignant angiogenesis was abated in a MC-deficient (KITW/KITWW_v) HPV16 transgenic mouse [97]. Tumor growth in this model was B cell-dependent [117] and required antibody deposition in premalignant skin [118] and activation of the Fcγ-receptors on myeloid cells [119]. This pioneering work has led to numerous reports that link MC with tumor angiogenesis and

reorganization of the tumor microenvironment [108]. Angiogenesis and tumor progression in a mouse model of breast cancer lung metastasis was shown to be CD4 T cell-dependent [120]. Inhibition of MC degranulation with cromolyn increased blood clotting and hypoxia in transplanted tumors growing in a mouse breast cancer model [121]. Inhibition of MC degranulation also suppressed tumor angiogenesis and tumor progression in a mouse model of Myc-induced beta cell pancreatic cancer [122].

We showed earlier that MC were the earliest detectable pro-inflammatory cells in aberrant crypts which developed in conditional mouse models of polyposis. Furthermore, hindering of the migration of bone marrow-derived MCP to the intestine dampened recruitment of myeloid cells to the intestine and severely attenuated polyposis [10]. Focal mastocytosis was TNF-α-dependent and neutralizing antibody to TNF-α-depleted mucosal MC as well as MCP causing loss of polyps in the small intestine [10]. Interestingly, focal mastocytosis in our mouse model of polyposis was independent of B cells and was exacerbated in immune-compromised Rag^{-/-} mice that were also deficient for the adenomatous polyposis gene. We later reported that MCP frequency in the small intestine is under the control of Treg and that adoptive transfer of Treg from healthy mice to mice with established polyposis depleted MCP and caused gradual regression of polyps in a time course-dependent manner [41]. In these studies, tumor growth was MC-dependent, and depletion of MC through genetic or pharmacologic means decreased tumor viability and increased apoptosis.

Other than promoting angiogenesis, MC also modulate hemostasis and blood perfusion in tumors. MC-produced heparin functions as a localized anticoagulant, and neutralization of heparin induced selective thrombosis of blood vessels within transplanted mouse tumors [123]. Little is known about other mechanisms by which MC promote tumor growth. Primary human lung MC were shown to be recruited to human thyroid tumors and induce thyroid cancer cell invasive ability, survival, and DNA synthesis *in vitro*; this observation was backed by demonstrating the ability of MC to promote tumor growth and invasion in a xenogenic mouse model. The MC effects were reported to be mediated by histamine and chemokines CXCL1/GROα and CXCL10/IP10 [124]. In a recent report, we provided evidence for MC 5-lipoxygenase (5-LO) activity contributing to tumor promotion. In particular, we showed that LTB₄ chemo-attracts myeloid-derived suppressor cells (MDSCs) and that 5-LO-deficient MC have a diminished ability to recruit MDSCs when compared with MC with the 5-LO enzyme [77]. Correspondingly, we observed a significant decrease in MDSC polyp infiltration with 5-LO deficiency. Our study demonstrated that hematopoietic 5-LO, and particularly MC 5-LO, promotes polyposis through its direct mitogenic effects on murine intestinal epithelium as well as through

its recruitment of MDSCs. We have also reported a novel mechanism through which MC promote inflammation and colon cancer that involves cross-talk of MC with Treg [41, 48], which is discussed in more detail later in this review.

6 Controversies on the protective role of mast cells in mouse models of cancer

Reports on the protective role of MC in mouse models of cancer are rare. One apparent exception is the MC-deficient compound mutant mice that were generated from the cross of the polyposis-prone Min mice to the KITW-sh/W-sh mice. Polyposis in these mice was more aggressive [125]. As already mentioned, MC mediate a variety of antimicrobial functions, and their total loss can be deleterious for the control of gut microflora (for review see [126]). In contrast, we have seen attenuated polyposis in MC-deficient APC^{Δ468} mice [10]. While the reason for the contrasting outcomes remains unknown, two potential causes deserve attention. It may be that lowered MC activity is protective while complete loss of MC is deleterious. Our mice were generated through reconstitution of the polyposis-prone APC^{Δ468} mice with bone marrow from KITW-sh/W-sh or CD34 and CD43 double knockout mice, which results in a profound decrease but not complete loss of MC in the chimeric mice. Indeed, the KITW-sh/W-sh mice suffer from chronic neutrophilia and gut inflammation. Additionally, the KITW-sh/W-sh defect is produced by chromosomal rearrangements that not only affect the c-Kit locus but also a number of tumor suppressor genes and oncogenes [127], presumably contributing to oncogenic events in this mouse strain, and this may be another confounding factor.

While the timely control of gut microflora by MC is protective, persistence of certain pathogens and MC will cause excessive and inappropriate release of inflammatory mediators, ending with pathologic consequences. For instance, *Shigella dysenteriae* stimulate intestinal MC to release excessive amounts of arachidonic acid metabolites including leukotriene C4 (LTC4) that then contribute to diarrhea and dysentery [128]. Arachidonic metabolites, in particular, those produced through cyclooxygenase 2 (COX2) activity have been linked etiologically with colon cancer [129, 130]. We recently reported that, in mouse models of polyposis, LTB4, a product of 5-lipoxygenase metabolism of arachidonic acid, critically contributes to polyp formation [77]. MC accumulation and degranulation in the mucosa are common features of gastritis in *Helicobacter pylori*-infected individuals [131–133]. Persistence of *H. pylori* is etiologically linked with human gastric cancer. Chronic infection of immune-deficient mice with *Helicobacter hepaticus* (the murine counterpart of this pathogenic bacterium) predisposes the mice to invasive

colon and mammary cancers [134, 135]. It remains to be seen whether deletion of MC in *H. hepaticus*-infected mice would hinder or exacerbate cancer incidence.

7 Association of mast cells with better clinical outcome in human cancers

There are reports of protective role for MC in human cancers. In a multivariate analysis of colorectal cancer patients including Dukes' stage, gender, age, peri-operative blood transfusion, tumor location, and counts of specific inflammatory cells, high counts of eosinophils, and MC predicted good survival [136]. MC tryptases activate the nuclear peroxisome proliferator-activated receptor- γ (PPAR- γ); expression of PPAR- γ is associated with better clinical outcome in colon cancer [137]. A subset of stage I non-small cell lung cancer patients had a worse prognosis at 5 years when low MC (tryptase–chymase phenotype) density was found in the peritumoral zone [113]. A study of 175 patients with non-small-cell lung cancer also demonstrated a correlation between improved prognosis and the presence of MC in islets but not surrounding stroma [138]. Tumor-infiltrating MC_T, after interleukin-2 preoperative induction therapy, were predictive of improved clinical outcome in patients with malignant mesothelioma, both for overall survival and time to progression [139, 140]. MC infiltration was reported to be a favorable prognostic marker in large B cell follicular lymphoma [141]. In contradiction to other reports already mentioned, Fleischmann and colleagues reported a positive correlation between MC infiltration of prostate tumors and better clinical outcome [142]. There are, however, differences in the types of MC that infiltrate tumors, and these differences may relate to different disease outcomes [143]. In benign human breast lesions, the number of MC exhibiting tryptase activity was similar to that of chymase-active MC, while malignant tumors had two to three times more tryptase-containing than chymase-containing mast cells [143]. Furthermore, presence of MC in the stroma of invasive breast cancers appears to correlate with a good prognosis [144, 145]. In prostate cancer, intratumor MC inhibit angiogenesis and tumor growth, while peritumor MC promote tumor growth [100].

8 Contribution of mast cells to tumor proliferation and invasion

MC have the capacity to promote tumor proliferation and invasion both directly by stimulating tumor cells and indirectly by modulating the tumor microenvironment. Apc^{Δ468} mice reconstituted with CD34^{-/-}CD43^{-/-} bone marrow are defective for MCP seeding of the tissues and

develop significantly less polyps in the intestine in comparison with the mice reconstituted with wild-type bone marrow [10]. Polyps in CD34^{-/-}CD43^{-/-} bone marrow chimeric mice had reduced mitotic index and increased apoptosis, in line with the slow growth of the lesions. In further studies, mouse MC-conditioned medium promoted proliferation of immortalized gut epithelial cells [77], and human MC-conditioned medium conveyed both mitogenic and invasive responses in cultured human pancreatic tumor cells [112].

Tumor cell mitogenic activity may be, in part, related to secretion of MC proteases. Tryptase-positive MC are abundant in the invasive front of human colon adenocarcinomas and are implicated in promoting tumor proliferation by activating protease-activated receptor-2 (PAR-2). MC tryptase cleaves and activates PAR2 on gut epithelial cells and myocytes [146, 147]. PAR-2 activation regulates gut motility [148], interrupts E-cadherin, and compromises epithelial barrier functions [149]. PAR2 activity also stimulates proliferation of colon cancer cells [150] and induces the expression of the prostaglandin-synthesizing enzyme COX2 and its metabolite prostaglandin-E2 [150], a potent immune-suppressive and tumor mitogenic soluble mediator. Overall consensus is that activation of PAR2 is an oncogenic event in colon cancer [151]. This conclusion is not limited to the GI tract, as PAR-2 activity is also considered critical for breast cancer migration and invasion [152]. Additionally, MC tryptases have been reported to elicit proliferative responses in airway epithelial cells [153], smooth muscle cells [154], and fibroblasts [155, 156]. MC tryptases also activate PPAR- γ that, in turn, promotes fibroblast proliferation [157].

Human MC chymases process the precursor IL- β into active IL-1 β , presumably in a proteasome-independent manner [158]. MC chymases modulate vascular tissues through their ability to process angiotensin-I to angiotensin-II [74] and are also involved in controlling gut epithelial barrier permeability [159, 160], a determining factor of gut inflammation. Other MC-derived factors that have mitogenic properties include heparin [161] and TNF- α . TNF- α is elevated in mouse models of polyposis, and its antibody-mediated neutralization attenuates disease [10, 135]. TNF- α has been also proposed to be tumor-promoting in human cancers [162].

Various studies have shown increased numbers of MC at the invasive border of tumors [17]. This, in part, reflects the role of MC in degrading the extracellular matrix and promoting angiogenesis, in preparation for tumor invasion. MC are rich sources of proteases [163–165]. Many of these can degrade stromal proteins. These include chymases and tryptases [163, 164], collagenases [166], MMP9/gelatinases [167–170], and cysteinyl cathepsins [171, 172]. Tryptases contribute to tissue remodeling through selective proteolysis of matrix proteins, activation of matrix metalloproteinases (MMPs) and modulation of blood supply to the tumor. Human MC β -tryptase possesses gelatin and collagen type I-degrading properties,

stimulates breast cancer cells to increase the release MMP-2, and promote subsequent *in vitro* migration and invasion of breast cancer [173]. Tryptases can stimulate the synthesis and release of collagen from fibroblasts in culture, as well as provoke secretion of collagenase [174]. Moreover, tryptases cleave fibronectin and type VI collagen, activate the pre-enzyme forms of some MMP and urinary plasminogen activators [126]. MC are potent inducers of fibrosis and stimulate fibroblast and myofibroblast proliferation [175, 176] to reorganize the tissue and shape a receptive tumor stroma.

These observations support the view that MC have a major role in promoting tumor cell proliferation and degrading the extracellular matrix, hence facilitating tumor growth and dissemination.

9 Mast cells present antigens and mobilized T-adaptive immune responses in cancer

MC are antigen-presenting cells, promote migration, maturation, and function of dendritic cells, and interact with T and B cells. Much is known about the MC orchestration of T cell responses [177–181]. MC-derived histamine, chemokines, LTB4, and TNF- α promote dendritic cell migration [182, 183], and lymphocyte recruitment [184–186], and hypertrophy of antigen-draining lymph nodes [184, 185, 187]. Recruitment of Treg into tumors is at least, in part, in response to MC-released adenosine [38]. MC are stimulated through contact with the recruited conventional CD4⁺ T cells and Treg to produce pro-inflammatory cytokines; this involves selective increase in Fc ϵ RI-mediated Stat5 phosphorylation, which is a critical mediator of IgE-mediated cytokine secretion [188].

MC express MHC class I and II molecules and OX40L upon activation and Notch signaling [189], and process and present antigens [190]. MC also express co-stimulatory molecules of the B7 family and members of the TNF and TNF receptor families, CD28, and CD40 ligand [191]. Activated MC release TH2 cytokines namely IL-4, IL-5, IL-9, and IL-13 that polarize T and B cell responses. MC are implicated in human TH2-driven IgE-associated food allergy, rhinitis, urticaria, angioedema, atopic dermatitis, and bronchospasm [126]. MC are held responsible for enhancing pathogenic TH1 cell responses in mouse models of autoimmune encephalomyelitis [192, 193]. Antigen presentation by MC in the context of MHC-II preferentially expands Treg [194].

10 Mast cell interaction with Treg determines outcome of cancer

MC or Treg infiltration of tumors correlates with favorable clinical outcome in some studies and worse prognosis in

others. MC have an intricate interaction with Treg that determines the functions of both cell types in a reciprocal manner (Fig. 2). The fate of this interaction dictates the level of cancer-associated inflammation and can enhance or suppress tumor growth.

Treg inhibit MCP and mature MC at multiple levels. Treg derived from healthy mice suppress MCP expansion and differentiation and MC degranulation [41, 48, 195], as well as expression of Fc ϵ RI and IgE-mediated LTC4 production [188]. Cell contact between Treg and MC is through OX40 and MC OX40L [48, 195] and, at least, for suppression of Fc ϵ RI, is IL-10- and TGF β -independent [188]. OX40L engagement on MC activates phospholipase C [196], elevates intracellular cAMP levels [197], and blocks the influx of Ca²⁺ needed for degranulation [195] (Fig. 3a).

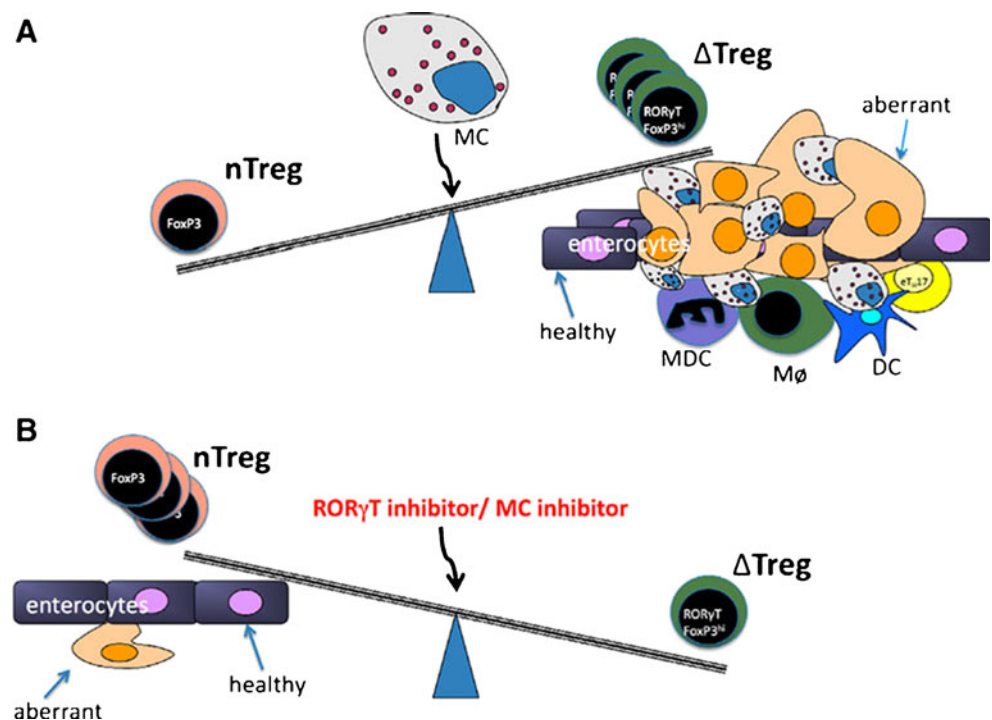
The interaction is reciprocal and has long-term repercussions for Treg. MC-derived histamine [198] and pro-inflammatory cytokines including IL-6 and IL-23 together with OX40 engagement on Treg abolish Treg-suppressive functions [41, 199–204]. MC can inhibit expression of IL-10 by Treg as well as by intraepithelial lymphocytes through OX40L–OX40 interaction [199, 200]. Through the same interaction, MC can commit Treg to TH17 lineage [203, 204] or generate pro-inflammatory Treg (Δ Treg) [41, 202]. Generation of both TH17 and Δ Treg requires expression of the orphan nuclear retinoic acid receptor-related orphan receptor- γ T (ROR γ T). However, Δ Treg differentiation is distinct from the classical TH17 conversion, as the Δ Treg

maintain expression of Foxp3, and their ability to suppress effector T cells do not produce IL-2 but are dependent on IL-2 for their survival and proliferation [48]. Δ Treg expand in mice with polyposis and in colon cancer patients, primarily in the pre-neoplastic lesions and tumors but also systemically in the lymphatics and blood [41, 48]. Elevated levels of IL-2 in colon cancer [48] may contribute to constraining TH17 cell differentiation of Treg [205, 206], in spite of the strong systemic TH17 cytokine bias in colon cancer patients [48]. The intact T cell-suppressive properties of Δ Treg contribute to tumor-specific immune tolerance while their pro-inflammatory properties help propagate tumor growth and dissemination [48].

Co-culture of Treg freshly isolated from healthy mice with *ex vivo* differentiated MC in medium containing SCF, and IL2 is sufficient to cause shut down of expression of IL10 and up-regulation of expression of IL17 by Treg in 5 days [48]. Since MC expand in polyps and tumors of the gastrointestinal tract, it is tempting to suggest that MC turn the tide in favor of cancer progression by recruiting and then altering the functions of Treg to promote further cancer-associated inflammation.

Loss of expression of IL-10 is a hallmark of change in Treg properties from anti-inflammatory to pro-inflammatory phenotype. Treg have potent anti-inflammatory properties that help protect the gut against bacteria-induced inflammation. IL-10-deficient Treg are unable to control gut inflammation [207–209], and IL10-deficient mice are prone to chronic bowel inflammation and cancer [210, 211]. Loss of IL10 has

Fig. 2 MC interaction with Treg modulates function by both cell types. **a** MC alter Treg properties and promote differentiation of pro-inflammatory and tumor-promoting Δ Treg. **b** Balance between anti-inflammatory and pro-inflammatory Treg determines the fate of tumor. **b** MC and expression of ROR γ T by Treg are critical for gain of pro-inflammatory properties by Treg. Blocking MC or ROR γ T will help maintain a protective anti-inflammatory state that disfavors tumor growth



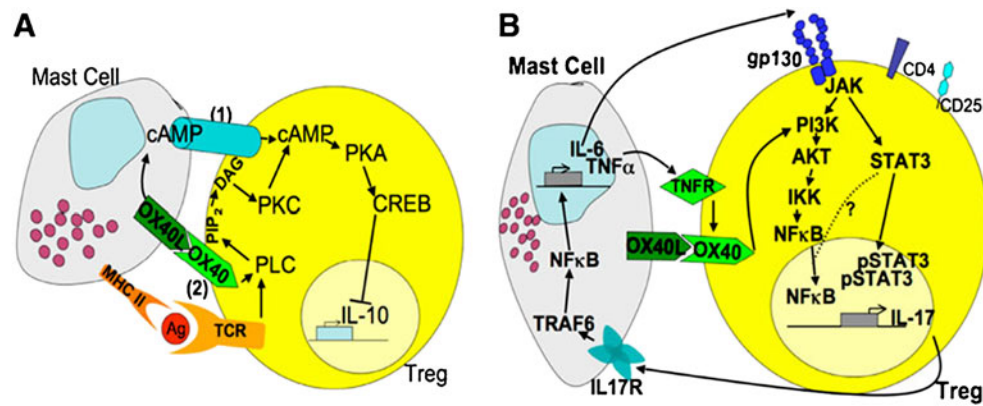


Fig. 3 Proposed mechanisms of diverting Treg to a pro-inflammatory phenotype. **a** OX40L–OX40 interaction with MC leads to increases intracellular levels of cAMP and shut down of IL10 gene expression

by Treg. **b** TNF α produced by MC upregulates OX40 in Treg and activates the PI3K pathway, while MC IL6 works through gp130 to stimulate the JAK–STAT3 pathway, allowing expression of IL17

profound consequences for the Treg. In the absence of IL10, the transcriptional factor ICOS preferentially promotes expression of IL17A, a potent pro-inflammatory cytokine [212]. Polyposis and colon cancer are driven by TH17 cytokines [41, 48]. Thus, loss of anti-inflammatory properties of Treg and deregulated production of TH17 cytokines has dire pathologic consequences. Uncontrolled inflammation fuels the growth and dissemination of colon cancer. Treg and MC are both pathologically implicated in autoimmunity and allergy, where in the absence of mutations that predispose to cellular transformation, MC-induced loss of Treg anti-inflammatory properties contributes to autoimmunity.

It is not known what cell type gives rise to pro-inflammatory Δ Treg. It remains to be shown whether these cells are generated from naturally occurring Treg (nTreg) or differentiate from helper CD4⁺ T cells in a process akin to that which generates iTreg. Also, it is not clear whether their expansion in cancer reflects the proliferation of a pre-existing population or *de novo* differentiation.

Co-expression of Foxp3 and ROR γ T and/or IL-17 has been reported in subpopulations of mouse Treg. In one study, the cells expressed IL-10 and CCL20 and suppressed T cells, and their ratio relative to regular Treg remained remarkably constant during infection and inflammation [213]. Similarly, cells expressing Foxp3 and IL17 have been detected in healthy human donors [214]. Activated human Treg as defined by Sakaguchi and colleagues contain a subpopulation that co-expresses IL17 [215]. Treg are endowed with plasticity and can readily differentiate into TH17 cells when activated in a permissive cytokine milieu [216–218]. Unlike undecided Treg that may be transitioning to TH17, activated Treg that co-express ROR γ T have higher levels of Foxp3 than naive nTreg. It remains to be seen whether these cells require activation and proliferation in order to differentiate into a pro-inflammatory phenotype, as in *ex vivo* MC co-culture with

Treg. Thus, potentially antagonistic pro-inflammatory and regulatory Treg co-exist and are normally tightly controlled through their interaction with MC, and perturbation of their equilibrium leads to chronic inflammation and carcinogenesis. This notion raises the possibility that, by targeted intervention, be it by adoptive transfer of healthy Treg, inhibition of MC, or by pharmacologic intervention in Treg properties, the anti-inflammatory properties of Treg in cancer can be recovered. This will predictably have devastating impacts on the tumor microenvironment and favor better clinical outcomes.

11 Concluding remarks

The hematopoietic system nurtures and feeds newly arising solid tumors, and MC appear to have a central role in this pathologic developmental process. Long known for their antimicrobial functions and role in mobilizing inflammation in wound healing, allergy, and autoimmunity, MC are indispensable in orchestrating innate and adaptive immune responses to promote cancer. The role of MC in normal physiology is poorly understood, but, based on their vicinity to stem cell compartments, can be speculated to be tissue remodeling. The early and abrupt appearance of intraepithelial MC in intestinal aberrant crypts only emphasizes a direct communication between MC and pre-neoplastic epithelial cells. Simultaneously, they orchestrate inflammatory reactions and angiogenesis that shape the tumor microenvironment and promote tumor cell proliferation and invasion. The tumor-promoting function of MC has been demonstrated in several experimental models, while its protective role in cancer remains controversial. The caveat is that all cancers are not the same, and the role of MC needs to be individually evaluated and established for each type of cancer and also for different stages of the same cancer.

Absolute MC deficiency (as in the cross of Min mice to the KITW-sh/W-sh mice) can have overarching immunological consequences. In the case of colon cancer, one could envisage that this will unleash microorganisms at the mucosal surfaces, drive pathogenic inflammation, and promote aggressive polyp growth. Treg fine-tunes MC functions to allow for control of pathogens and at the same time prevent excessive tumor inflammation. However, in the cancer setting, MC promote a gradual gain of pro-inflammatory properties by Treg favoring uncontrolled escalation of cancer inflammation, tumor-immune tolerance, and aggressive tumor growth. Therapeutic strategies targeting the tumor microenvironment and host response should aim to restore healthy immune interactions, rather than attempt elimination of a major cellular compartment. In this review, we have emphasized the interaction of MC with Treg as a potential novel target for therapeutic intervention in colon cancer.

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