

Chapter 6

Mast Cells in Allergy and Tumor Disease

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Abstract In allergy, mast cells (MCs) are known for the notoriously detrimental role they play. MC mediators like histamine, prostaglandins, leukotrienes and thromboxane are released during the early phase of an allergic reaction and produce inflammation and local tissue responses. One pervasive roadblock in the effective development of therapeutic immunity to cancer is the fact that tumors create immune privileged sites that are resistant to the development of effective inflammation and effective anti-tumor responses. In this chapter we will present the current knowledge of the inflammatory responses elicited in allergies by IgE and MCs as well as the current knowledge on how the responses of MCs may regulate tumor growth and the immune response to tumors.

6.1 Introduction

There are a few, if any, inflammatory responses that are more rapid or intense than those mediated by IgE. IgE exerts its massive and sometimes deadly pro-inflammatory effects by triggering the release of a vast array of pro-inflammatory mediators from mast cells. MCs, first termed by Paul Ehrlich in his graduate thesis, are best known for their protective role in helminth infections and allergy [1]. During helminth infections, MCs accumulate in numbers (mastocytosis) at the site of infection (skin, intestines and lung), eosinophils are recruited, there is an induction of goblet cell hyperplasia, high levels of IgE are elicited and a Th2 inflammatory environment is created, leading to the development of protective immunity. In allergy, MCs are known for the notoriously detrimental role they play. MC mediators like histamine, prostaglandins, leukotrienes and thromboxane during the early phase of an allergic reaction are released and produce local tissue responses. In the respiratory tract, the classic symptoms of sneezing, edema and mucus secretion

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are evident, with vasodilatation in the nose and bronchoconstriction in the lung. As allergies proceed in the lung, there is accumulating cellular infiltration, fibrin deposition and tissue destruction as a result of a sustained allergic response leading to edema and further inflammatory cell recruitment. Immunologically, helminth infections and allergies show striking similarities such as induction of Th2 responses, eosinophilia, goblet cell hyperplasia, mastocytosis and high levels of IgE [2]. However, helminth infection and allergy seem to be mutually exclusive as has been shown by several epidemiological studies [3–5].

One pervasive roadblock in the effective development of therapeutic immunity to cancer is the fact that tumors create immune privileged sites that are resistant to the development of effective inflammation. Given this fact and the extraordinary capacity of IgE to mediate intense inflammatory responses a series of studies starting in the 1950s were undertaken to induce allergic reactions against tumors [6]. This novel approach in the context of the newly emerging field of AllergoOncology, the use of “allergy” as anti-tumor therapy, was later strengthened by epidemiological studies that show a protective effect of the presence of IgE on the development of malignancies [7–10]. Given the state of advanced antibody engineering, the widespread use of therapeutic antibodies and the ease with which one can produce fully human tumor-specific antibodies, IgE monoclonal antibodies directed at tumor antigens can be vigorously tested for their therapeutic value. In this chapter we will present the current knowledge of the inflammatory responses elicited in allergies by IgE and MCs as well as the current knowledge on how the responses of MCs may regulate tumor growth and the immune response to tumors.

6.2 Mast Cells in Allergy

6.2.1 *Allergies and Mast Cell Subsets*

In non-atopic individuals, B cells are skewed toward the production of allergen-specific IgG4 or IgA and to lesser extent IgG1, whereas in atopic patients B cells are driven to switch to IgE production [11]. However, IgE production is not a prerequisite for the development of disease, as allergy can be either IgE or cell (non-IgE) mediated or a combination of both. Although allergies are multifaceted, the involvement of MCs is considered one of the hallmarks in both mouse models and humans, and depending on the model, MCs have been proven to be the critical effector cells in allergy [11, 12]. MCs are divided into two subsets based on location and content: mucosal (MMC) and connective tissue (CTMC) MCs. These subsets can be distinguished based upon the presence of proteolytic enzymes in the granules. MMCs contain both chymases, chymotryptic peptidases, and tryptases, serine-class peptidases, whereas CTMCs only contain chymases [12]. Activation of MCs after allergen encounter leads to the release of chymases among many other mediators and does not seem to be dependent on subset. Chymases are mainly involved in

tissue remodeling [13], and thus MCs degranulation could exert a profound impact on the tumor microenvironment.

MMC and CTMC may be involved in Th2 versus Th1 responses, respectively. MMC and CTMC differ in the expression of STAT4, in that the latter express STAT4 and may play a role in driving Th1 responses. These findings suggest that CTMCs might not be prominently involved in Th2-driven allergies [14]. STAT4 seems to be dispensable for Th1 skewing in T cells, but the presence of STAT4 in CTMCs leads to the production of interferon gamma (INF- γ) and nitric oxide (NO) and is involved in the response to interleukin-18 (IL-18), IL-4, and IL-13. Additionally, Th1 skewing by CTMCs shows increased tumor cell lytic activity [14, 15]. The contrast of CTMCs with MMCs is even more striking when both subsets get triggered by IL-18, a cytokine that synergizes with IL-12 to induce cell-mediated immunity. CTMCs produce INF- γ when stimulated with IL-12 and IL-18, the same treatment elicits the production of IL-4 and IL-13 in MMCs, hallmark cytokines of IgE-mediated allergy [14, 16]. However, a strict demarcation of MC subsets into mediating Th1 versus Th2 responses cannot be delineated based on the current definitions of CTMCs versus MMCs. Allergens can enter the body through ingestion (food allergies are mainly MMCs mediated), inhalation (allergic rhinitis and asthma are mediated by both CTMCs and MMCs) or via the skin (atopic dermatitis is mainly CTMCs mediated) suggesting that both subsets play a role in allergy. It is likely that there are additional phenotypic distinctions among MC subsets that would further delineate them into MCs that play a role in Th1 versus Th2 responses. No doubt, it is critical to develop a more comprehensive understanding of the functional and transcriptional profile of subsets of MCs if they are going to be exploited to mediate anti-tumor immunity.

6.2.2 Mast Cells and Dendritic Cells

There is little doubt that the presentation and cross-presentation of tumor antigens by dendritic cells (DCs) is essential for the development of tumor-specific CD4⁺ and CD8⁺ T cells and protective anti-tumor immunity. Emerging data illustrate that MCs play an early role in DC migration and function, and it is in this context that MCs may contribute to the development of tumor immunity. Contact hypersensitivity (CHS) was regarded a Th1 cell-mediated allergy and has been studied extensively [17]. However, Th2-dependent CHS has been reported and seems to be dependent on allergen [18, 19]. One of the important early events in the development of CHS is the degranulation of MCs leading to the release of vasoactive and inflammatory mediators, like IL- α/β , histamine, tumor necrosis factor (TNF)- α , leukotrienes and granulocyte-macrophage colony-stimulating factor (GM-CSF) [20–22]. It has been shown that these mediators can dramatically modulate DC maturation and induce DC migration. Especially in Th2-driven CHS, TNF- α plays a crucial role in controlling DC migration [23]. Late phase disease is characterized by the infiltration of the skin by MCs, local swelling and recruitment of antigen-specific T cells, and

other leukocytes, to the site where the hapten is applied [24–26]. Additionally, the role of MCs in CHS has been refined very recently and even though the disease still develops in the absence of MCs, this study showed that MCs are regulators of the magnitude and the cytokine microenvironment [27]. Many insights on the interplay between MCs and DCs/Langerhans cells (LCs) come from CHS studies.

As mentioned earlier, TNF- α plays an important role in the development of CHS and can be produced by MCs in soluble and membrane-bound “cell-associated” form [28–30]. TNF- α is necessary for optimal response at the site where the hapten is applied during challenge [28]. Additionally, MC-deficient mice show impaired migration of LCs to the draining lymph nodes (dLN), a delay of approximately 18 h was observed while activation of MCs by IgE and allergen showed enhanced migration of LCs from the epidermis [31, 32]. The two observations were elegantly fused to show that cell-membrane-associated TNF- α produced by MCs regulating DCs/LCs migration, both in the skin and in the lung [33]. This implies that under atopic conditions, MCs modulate the adaptive immune responses by triggering increased antigen presentation in the dLN through enhanced DC migration.

There are TNF- α -independent ways MCs promote DC/LC migration. Patients with atopic dermatitis (AD) and CHS show a diminished defense against pathogens by reduced amounts of antimicrobial peptides (AMPs) and increased permeability of the skin by delayed repair [34–36]. Therefore, the lesions formed during encounter with the allergen are confronted with a wide array of Toll-like receptor (TLR) activation signals. It is known from studies with TLR2 agonist *Staphylococcus aureus* peptidoglycan (PGN) that MCs can induce LC migration equally well in wild type (WT) or TNF- $^{-/-}$ mice [22]. The authors concluded that this was TLR2/MyD88 independent but complement dependent, which is plausible since PGN can activate complement efficiently. This observation could implicate that PGN leads to IgE-independent complement-dependent degranulation since IgE/antigen-mediated degranulation yielded the same results although the latter is dependent on TNF- α [22, 37]. Another study shows that MC cells up-regulate IL-1 β when activated with imiquimod, a TLR7 agonist. Even though there was a trend suggesting involvement of TNF- α , there was a significant reduction in LC migration in IL-1 α/β - $^{-/-}$ mice [38]. MCs also express a functional TLR4 receptor [39, 40]. Upon infection of gram-negative bacteria MCs respond by the release of TNF- α as the main pro-inflammatory cytokine, thereby promoting DC maturation and migration [39]. Additionally IL-1 β , IL-6 and IL-13 are produced and in the presence of IgE-mediated degranulation as seen in allergies IL-5 and IL-10 are released as well, thereby creating a Th2 cytokine environment [41]. These studies clearly show that multiple different responses to infection during CHS or AD can lead to aggravation of disease that is independent of the allergen but dependent on MCs which are abundantly present.

Another mechanism by which MCs influence DC function is through indirectly modulating the allergic microenvironment. It has been shown that MCs regulate thymic stromal lymphopoietin (TSLP) expressed by epithelial cells. TSLP activates DCs and lead to the induction of Th2 cells from naive CD4 $^{+}$ T cells [42, 43]. It has been shown in a murine allergic rhinitis model that allergen challenge leads to

upregulation of TSLP in a MC- and IgE-dependent manner, thereby contributing to the Th2 environment and enhanced DC migration and maturation [44].

These modulatory properties imprinted by MCs on DCs are reflected in the development of the subsequent T-cell responses. In allergy models, it has been shown that MCs induce a Th2 profile by the production of PGE2 and histamine. These mediators induce DCs to produce CCL17/22, which are Th2-cell recruitment factors and also suppress the frequency of Th1 allergen-specific cells both in vivo and in vitro [45–47]. This further emphasizes the important role of MCs during allergic responses. Altogether, the current understanding is that there is an intimate and complex network of communication between MCs and DCs, which can exert profound effects on the development of acquired immunity.

6.2.3 Mast Cells and T-Cells

Although MCs indirectly influence T-cell responses via DCs, there is a body of information that shows MCs may also directly impact the T-cell compartment. During allergen encounter, IgE-sensitized MCs degranulate and release a wide array of mediators in the environment. Many of these factors affect the migration, development, proliferation and phenotype of T cells as has been reviewed extensively. As such, this represents another way in which MCs can orchestrate the development of the adaptive immune response [48–52]. The observation that MCs and T cells can be found in close proximity to each other suggests that cell-to-cell contact and/or regional cytokine production play key roles in this interaction [53]. Although MCs are not present in high numbers in secondary lymphoid organs, under inflammatory conditions, like during allergic reactions, MCs migrate from the periphery to the regional node [54, 55]. This suggests that MCs can interact with T cells both in tissue and in lymphoid organs. Additionally, since MCs express MHC-I and MHC-II, a role as antigen-presenting cells has been suggested and in fact demonstrated in vitro [56–58].

The idea of MCs acting as antigen-presenting cells (APCs) is not a novel concept. In 1993, Frandji et al. observed that MCs can activate T cell in vitro in an antigen-dependent manner leading to T-cell proliferation [56]. Yet even 10 years earlier, it was reported that MC progenitors could up-regulate MHC-II in the presence of IFN- γ [59]. Paradoxically, IFN- γ leads to the down-regulation of co-stimulatory molecules CD80 and CD86 on MCs [60]. More recently, MCs have been shown to express other co-stimulatory molecules of both the TNF-super family (OX40L, CD30L, Fas and GITR) as well as the B7 family CD80, CD86, programmed death ligand (PD-L)1 and PD-L2 [61]. IL-4 up-regulates MHC-II, albeit less than with IFN- γ but in combination with the up-regulation of co-stimulatory molecules [62]. Additionally, IL-4-cultured MCs but not IFN- γ -cultured MCs induce T-cell proliferation in vitro [52, 60]. These results suggest that MCs can enhance Th2 responses by positive feedback of IL-4 but dampen Th1 by negative regulation of IFN- γ .

Engagement of TLR4 on MCs induces MHC-II expression, and MCs can phagocytose, process and present bacterial antigens in the context of MHC-I [58]. All

these studies clearly show that MCs can be functional APCs; however, whether the same occurs in vivo is not yet clear. Since it appears that MCs undergo a cytokine-dependent up-regulation of MHC molecules it is feasible that the antigen-presenting function of MCs is to activate T cells locally in the peripheral tissues, where pro-inflammatory cytokines are released.

Although the role of MCs as *bone fide* APCs in vivo is debatable, antigen-independent regulation of T-cell response has been widely studied and recognized. The release of a wide array of immune-modulatory mediators induces recruitment, activation, skewing and proliferation of T cells. When activated, MCs release their granular content with the first effects being thought to involve recruitment of T cells and other leukocytes from the blood. The release of soluble TNF- α leads to up-regulation of several adhesion molecules, such as ICAM1, VCAM1 and VAP1, on endothelial cells needed for extravasation of T cells from the blood to the site of inflammation [63, 64]. Although seemingly contradictory, TNF- α also induces T-cell migration to the dLN during infection [65]. In this context, we speculate that TNF- α may function as a gatekeeper leading the T cells through the site of inflammation by recruiting them from the blood and directing them toward the dLN. Another factor known to promote extravasation is histamine. During allergies, the release of histamine increases the permeability of the vasculature, thereby increasing the efflux of T-effectors among other leukocytes [66].

A long list of chemokines can be released to fine tune the inflammatory response locally [51, 67, 68]. Once the T cells reach the location of allergen challenge, the release of IL-4, IL-13 and IL-10 by MCs sets the stage for a Th2 response [68]. Although not always the case, it seems that MCs preferentially skew toward a Th2 response. For instance histamine can both promote Th1 responses or suppress Th2 and Th1 T-cell activation depending on the receptor engaged [69].

In addition to the role of MCs during allergic inflammation by their influence on the adaptive immune response MCs have a well-established innate immune function and play an important role in wound healing and in the eradication of nematodes. These innate functions are also revealed during adaptive immune responses as tissue remodeling and destruction.

6.2.4 Mast Cells and Airway Tissue Remodeling

MCs products can directly affect airway epithelium and airway smooth muscle (ASM) function to enhance airway inflammation and hyperresponsiveness. Persistent MC activation during asthma can also lead to airway remodeling. This process is associated with damage and aberrant repair to lung tissue, including epithelial damage both at the surface of the lung and in the underlying basal lamina, increased development of myofibroblasts from fibroblasts, increased size and number of smooth muscle cells, and increased numbers of goblet cells (which produce mucus) [70]. One of the principle MC mediators of this process is β -tryptase, which enhances the proliferation of fibroblasts, airway epithelial and endothelial cells,

and smooth muscle cells, leading to global airway thickening [71–73]. Other products that contribute to airway remodeling are transforming growth factor (TGF)- β , which drives the differentiation of fibroblasts and ASM cells, and amphiregulin, which causes the proliferation of lung fibroblasts as well up-regulates mucin gene expression [74–76]. In addition, the MC products histamine, prostaglandin D₂ and leukotriene C₄ can cause ASM contraction [77, 78].

There is increasing evidence that angiogenesis plays an important role during tissue remodeling to provide oxygen and nutrients to the inflamed tissue as well as to allow the influx of inflammatory cells to the site. The most compelling evidence for this is that increased vascularity of the airways is observed in patients with both severe and mild asthma in comparison to healthy controls [79–82]. MCs derived IL-4, IL-5, IL-13 and TGF- β that can induce the production of vascular endothelial growth factor (VEGF) by fibroblasts and ASM cells contributing strongly to neo-vascularization [83, 84]. In addition, VEGF can to some extent regulate immune responses by inhibiting DC maturation and serve as a chemoattractant for other lymphocytes [85, 86].

6.3 Mast Cells in Cancer

6.3.1 Introduction

It has been proposed that the infiltration of MCs at the tumor site can greatly enhance tumorigenesis; MC accumulation has been confirmed in both experimental models in rodents and in primary human tumor biopsies; and MC accumulation in tumors is often associated with a poor prognosis [87, 88]. It has been suggested that MCs contribute to enhanced tumor growth by enhancing tumor-associated angiogenesis and by facilitating tissue remodeling. As noted above, MC production and induction of pro-angiogenic factors, as observed in asthma, may support the creation of a supportive environment for the growth of tumor cells. Likewise, the same mediators responsible for tissue remodeling in asthma may help to degrade the extracellular matrix and facilitate tumor growth and metastasis. Furthermore, MCs may enhance tumor growth through the release of tumor growth factors and by means of suppressing the development of protective anti-tumor immunity.

6.3.2 Mast Cells and Angiogenesis

Several factors underscore the importance of vasculature and angiogenesis for neoplastic growth and spread [89]. The original observations that tumor-associated vasculature plays an important role in the growth of neoplasms were made by Goldmann in 1907 [90]. Half a decade later (1970), a positive correlation of tumor growth and induced vasculature became evident. During the early stages of tumor development, tumors need oxygen and nutrients for continued growth and differentiation. Excessive tumor growth creates a hypoxic environment which in turn

promotes new blood vasculature through the up-regulation of angiogenic mediators released by tumor as well as non-tumor cells.

The impact of MCs on tumor angiogenesis was initially described in 1988 when it was shown that the angiogenic response was impaired in mast cell-deficient (W/W^v) mice in comparison to WT mice. In these studies, fewer W/W^v mice than WT mice developed spontaneous lung metastases and W/W^v mice exhibited fewer lung metastases per mouse. Bone-marrow reconstitution of W/W^v mice restored the angiogenic responses and also the incidence of hematogenous metastases to that of WT mice [91]. These studies implicated a role of MCs *in vivo* for tumor angiogenesis. Later Coussens and co-workers demonstrated that early angiogenic activity was greatly reduced in W/W^v mice using the HPV16 transgenic mouse model of squamous cell carcinoma, showing that up-regulated expression of the matrix metalloproteinase (MMP)-9 coincided with the angiogenic switch in early tumor lesions [92].

Recent studies confirmed these earlier observations establishing that MCs are an essential hematopoietic component for pre-neoplastic polyp development. It was seen that from the onset, adenomatous polyps are infiltrated with MCs and their precursors. Depletion of MCs either pharmacologically or by using MC-deficient animals resulted in a profound remission of existing polyps [93]. In addition, MCs have been shown to be necessary for the angiogenesis and macroscopic expansion of c-Myc-induced pancreatic β -cell tumors [94].

MCs have been shown to be selectively recruited to the site of tumor growth by means of tumor-derived peptides, RANTES and MCP-1 [95, 96], and several important angiogenic factors are expressed and secreted by MCs, such as IL-8, IL-1, VEGF, fibroblast growth factor-2 (FGF-2), TGF- β , TGF- α , MMP-2, MMP-9, nerve growth factor (NGF), platelet-derived growth factor (PDGF), leptin, tryptase, chymase, histamine and heparin [97].

IL-8 acts both as an angiogenic factor as well as a tumor cell chemotactic factor and tumor mitogen [97]. Inhibition of IL-8 by neutralizing antibodies reduces human non-small cell lung carcinoma progression in mice [98].

IL-1 secreted by MCs contributes to neovascularization by stimulating the secretion of VEGF from stroma surrounding tumor as well as directly acting on tumor growth [99]. In addition, IL-1 can induce selective secretion of IL-6 without overt degranulation in cultures of human MCs. High levels of circulating IL-6 are observed in almost every type of tumor studied and predict a poor patient outcome [100].

MC MMPs contribute to tumor metastases and invasiveness due to their proteolytic activity [101]. MC MMP products, for instance, have been seen to contribute to brain tumor metastases by regulating the permeability of the blood-brain barrier [102].

Currently MC involvement in tumor growth by means of neovascularization is relatively well accepted due to studies in murine tumor models. Below we will review observations linking MCs and tumor growth from human studies and will further elaborate on potential roles of MCs on tumor growth.

6.3.3 Mast Cells in Human Tumors

Some of the most aggressive human tumors, including breast carcinoma, malignant melanoma and colorectal adenocarcinoma, are notably populated with MCs at their periphery [87, 88, 103]. MCs have been found in the connective tissue surrounding tumor and in the environment of lymphatic and blood vessels. In mammary tumors, MCs are found on the invading, growing front of the tumor, and they contain elevated tryptase expression as compared to MCs in benign lesions.

Similar observations have been made about MCs in renal tumor tissue, and increased MC density correlates with poor prognosis in non-small lung carcinoma and increased VEGF expression [104]. Increased VEGF expression found in clinical samples may be the basis for the studies reported by Norrby and Wooley in 1993 [105], demonstrating a positive correlation of MCs with increased tumor angiogenesis. In the 1990s, a closer correlation was seen between angiogenesis and MCs in several different tumor models, such as angioma, lymphoma and multiple myeloma [106]. In Table 6.1 we summarize several important mouse and human studies investigating the connection between MCs and tumor and list the major outcomes of the studies.

MCs are represented throughout the literature as contributing to tumorigenesis and poor prognosis. In tumor specimens from Hodgkin's lymphoma (HL), where sections were stained with MC-specific antibody that detects tryptase, MCs were seen in virtually every case. Furthermore, higher MC infiltration correlated with increased tumor aggressiveness [107]. If there is a case to be made for MCs contributing to enhanced tumor growth and metastasis, then lessening the infiltration or function of MCs in tumors might be an attractive therapeutic approach. In a study of advanced human breast carcinoma it was seen that recombinant human stem cell factor (SCF) (c-kit ligand) promoted human MC activation *in vivo*. As such, neutralization of c-kit ligand on MCs may represent a potential therapeutic target [108].

6.3.4 Mast Cells Mediators of Tumor Growth or Rejection

The link between inflammation and cancer has long been recognized and has been recently reviewed by Coussens et al. [109]. The inflammatory nature of the MCs may allow them to act as both beneficiaries of tumor growth and contributors of tumor rejection.

With the wealth of correlative clinical data, the early infiltration of MCs into human tumors appears to correlate with a poor prognosis. Given this evidence, animal cancer models have been used to try to understand whether MCs contribute to tumor growth and demise of the host, or in fact protect the host from cancer. Contrary to many human clinical observations, studies in one murine model have shown that MCs can exert a protective role for the host in a genetic model of intestinal tumorigenesis. This model of spontaneous intestinal adenomas in the

Table 6.1 Mast cell infiltration and role in tumor progression in human and murine tumor models

Tumor	Mouse model	Outcome of study	References
Murine, pro-tumor Mouse plasmacytoma	BALB/c nu/nu mice	Primary mast cells accelerate tumor growth through Ang-1 and VEGF-A	Nakayama et al. [127]
K14-HPV16 spontaneous murine model (models human cervical cancer)	(KitW/KitWW ^v) HPV16 transgenic mice	Mast cell infiltration and activation of MMP-9 coincides with the angiogenic switch in premalignant lesions. Premalignant angiogenesis is diminished in MC-deficient HPV16 transgenic mice	Coussens et al. [128]
b-cell carcinoma	plns-mycERTAM;RIP7- bcl-xL;KitW-sh;KitW-sh	Mast cells are necessary for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors	Soucek et al. [93]
Murine, anti-tumor Murine mammary adenocarcinoma cell line (4T1)	BALB/c mice	Imatinib mesylate (Gleevec) accelerated the growth and peri-tumoral blood clotting of mammary adenocarcinoma in mice by inhibiting the survival and function of MCs	Samoszuk et al. [129]
Human, pro-tumor MM	–	Angiogenesis parallels progression of MM and MC participate in the induction by release of angiogenic factors. Poor prognosis in MM correlates directly with increased MC number	Ribatti et al. [130]
Rectal cancer	–	In a study of 331 rectal cancer samples a correlation was found of worsened overall survival rate with increase in MC present around tumor	Fisher et al. [131]
Melanoma	–	A high correlation was demonstrated between microvessel counts and total metachromatic and tryptase-reactive MCs. Suggesting that tryptase secreted by MCs cooperate in angiogenesis induction in human melanoma and as such increasing tumor progression	Ribatti et al. [132]
Oral SCC	–	MCs in all lesions and normal mucosa express tryptase. Density of MCs and microvessels increase with disease progression	Iamaroon et al. [133]
Lung adenocarcinoma	–	MCs correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma	Imada et al. [134]
HL	–	MCs were detected in all tumor samples and increased number of MCs correlated with nodular sclerosis histology and a worse relapse-free survival	Molin et al. [106]

Table 6.1 (continued)

Tumor	Mouse model	Outcome of study	References
Pterygium	–	Angiogenesis was highly correlated with MC tryptase-positive cell count in pterygium tissues. Neovascularization in pterygium may be sustained by MC angiogenic mediators	Ribatti et al. [135]
Human, anti-tumor Breast, head and neck, lung, ovarian, NHL, HD	–	MCs are present in fibrotic regions of cancer and MC heparin is a inhibitor of tumor growth	Samoszuk et al. [136]
Breast carcinoma	–	A high number of MCs in tumor stroma were related to low S phase fraction, DNA diploidy, high proportion of intraductal growth and a high degree of tubule formation. A high MC count was related to a favorable prognosis	Aaltomaa et al. [137]
Breast carcinoma	–	Tissue microarrays of invasive breast carcinoma demonstrated the presence of MCs in the stroma correlating with a good prognosis. The presence of MCs showed an inverse correlation with CD68 positive macrophages and no correlation with the presence of B cells or T cells	Dabiri et al. [138]
Ovarian cancer	–	There was no significant association between angiogenesis and mast cell density. Peri-tumoral MC infiltration in tumors with high MVD predicts for improved survival in women with advanced epithelial ovarian cancer	Chan et al. [139]
Human, neutral RCC	–	The correlation between microvessels and MC counts was established and compared with tumor stage, grade and additional clinicopathologic parameters. No correlation was found between MC number and tumor size, stage, grade or patient survival	Tuna et al. [140]
Breast cancer	–	No difference in 10-year disease-free survival was established in patients with and without MC infiltration suggesting no correlation between MC numbers and prognosis	Fisher et al. [141]
NSCLC	–	Macrophage and MC infiltration of tumor cell islets confers a marked survival advantage in NSCLC	Welsh et al. [142]

Abbreviations: MM, multiple myeloma; SCC, small cell carcinoma; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; HD, Hodgkin's disease; RCC, renal cell carcinoma; NSCLC, non-small cell lung carcinoma; VEGF, vascular endothelial growth factor; MVD, mean vessel density

multiple intestinal neoplasia (Min, APC^{Min/+}) mouse was seen to have an increased number of MCs and increased abundance of MC-associated proteinases. The MC-deficient Min (Min-Sash) mice developed 50% more adenomas than littermate controls, and the tumors were 33% larger in Min-Sash mice. These results suggest a protective, anti-tumor role for MCs in a genetic model of early-stage intestinal tumorigenesis [110]. Indeed, many of the cytokines produced by MCs can conceivably lead to impaired tumor growth. For example, the IL-4 receptor (IL-4R) is expressed on human breast carcinoma and signaling through the IL-4R can lead to tumor cell apoptosis, allowing for the possibility that MCs may act in a protective role to the host by directly killing tumor cells via IL-4 or other apoptosis-producing cytokines like TNF- α . Additionally, TNF- α contributes to leukocyte infiltration into the tumor which may also be protective [29, 111].

However, the overwhelming evidence in animal models suggests that MCs facilitate tumor growth and progression. In mammary tumor development MCs allow for tumor angiogenesis, contribute to tissue remodeling and metastasis through the release of MMPs and the release of growth factors such as SCF and NGF [112].

Using the mouse model of MC deficiency of the Kit^W/Kit^{Wv} mice Galli et al. conclusively demonstrated that these mice have decreased susceptibility to the development of 1,2-dimethylhydrazine-induced intestinal tumors [113]. These results were later supported, as described above, by studies in spontaneous human colorectal cancers where MCs are essential for polyp development and play a protective role to the tumor in intestinal tumorigenesis [93, 110]. Furthermore, the same studies show that TNF- α is required for the growth of adenomatous polyps, and it is proposed that MC-derived TNF- α is acting in an autocrine loop as a MC recruiting factor to increase the concentration of MCs at the site. Figure 6.1 summarizes the multiple ways by which MCs may either promote tumor growth or contribute to tumor rejection.

6.3.5 Mast Cells Regulate Adaptive Immune Responses to Tumors

In addition to the generally accepted role of MCs influencing tumor growth by means of angiogenesis and tissue remodeling, MCs may exert an immunomodulatory role in the generation of anti-tumor immune responses. Tumors utilize several mechanisms by which they evade the immune response including the induction of T-cell ignorance and anergy and by means of active suppression of effector cells [114–118]. The idea that MCs are beneficial for tumor formation together with the fact that MCs can selectively release cytokines and other mediators without degranulation raises the question whether MCs contribute in the development of an immune privilege within the tumor microenvironment. Such selective release of MC mediators is called piece meal degranulation and is a critical factor in MC versatility and their ability to release certain products without others and without degranulation [119].

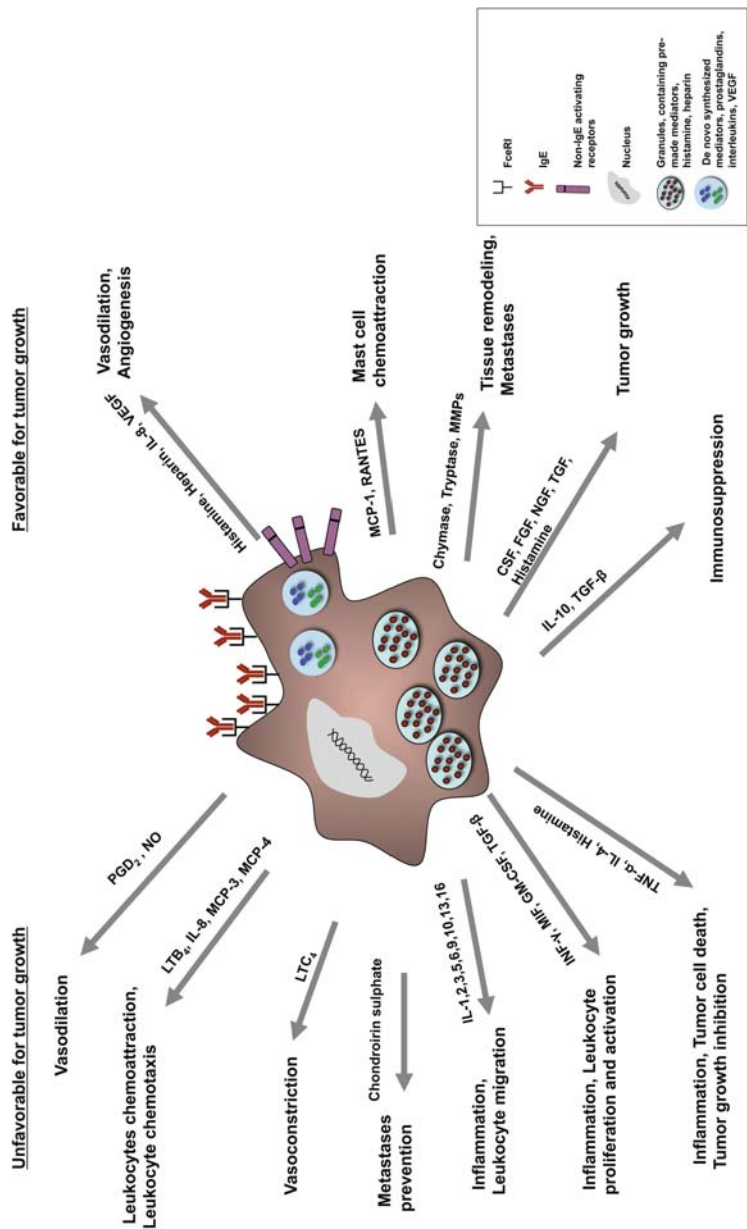


Fig. 6.1 Mast cell mediators and their effect on tumor growth. MCs carry and release multiple mediators that can both favorably and unfavorably affect tumor growth. Among the MC mediators thought to be unfavorable to tumor growth are those which cause leukocyte infiltration to the tumor site, vasoconstriction, prevent metastases, cause inflammation, cause leukocyte proliferation and activation and have direct anti-tumor effect by means of tumor cell death and tumor growth inhibition. Conversely, other factors, such as the pro-angiogenic mediators IL-8 and VEGF, are thought to contribute favorably to tumor growth by inducing angiogenesis; chymases and tryptases are known for their role in tissue remodeling and tumor metastasis and other factors such as FGF and NGF act directly on tumor growth. Factors such as IL-10 act in an immunosuppressive manner and yet others in the increased chemoattraction of additional MCs. What role MCs play during tumor progression likely varies depending on their immediate environment and the stimuli they encounter

Some of the immunoregulatory components of MC function are believed to be mediated by the release of histamine. Histamine released from MCs has been shown to play a dual role in that it can stimulate the proliferation of tumor cells through the H1 receptors (H1R) and act as a negative modulator of immune cells through H2 receptors (H2R) [112]. Results suggest that histamine in tumor tissue suppresses local tumor immunity via H2R activation that results in tumor growth promotion. In addition, and somewhat conflicting with the immune stimulatory role of signaling through H1R, histamine has been seen to enhance growth of melanoma in a H1R-dependent manner [120]. The effect of histamine directly on tumor growth is currently quite a conflicting topic as studies report differing results depending on dosage of histamine used as well as type of tumor studied. While it is likely that tumor-infiltrating MCs provide enough histamine to act as a tumor growth factor, exogenous administration of high-dose histamine seems to instead result in an anti-tumor effect of histamine in both a direct and an indirect manner [121].

MCs may contribute to tumor growth via immune modulation in the same way described above due to their interactions and influence on DC migration and functionality as well as by directly modulating T-cell responses. MCs produce IL-10, a common immunomodulatory cytokine produced by a variety of T-cells including T-regulatory cells (T_{reg}). Interestingly, MC-derived IL-10 has been shown to play a significant role in the suppression of UV-B-induced CHS [122]. MC-derived IL-10 is also seen to reduce immune responses after mosquito bites [123]. Hence, the production of IL-10 by MCs at the tumor site may contribute to the generation of an immunosuppressive microenvironment. In addition to providing immunosuppressive factors, MCs may also act in a suppressive way by removing proinflammatory mediators from the tumor microenvironment such as serotonin. Serotonin was recently shown to promote T-cell proliferation and activation, and MCs express the serotonin-specific transporter (SERT) by which they can take up and store serotonin [124–126].

As reviewed earlier, MCs can up-regulate the expression of several co-stimulatory molecules, and in addition it has been shown that MCs express PD-L1 and PD-L2, the ligands for the inhibitory receptor PD1 expressed on T cells [61]. These inhibitory ligands are expressed on tumors and tumor-infiltrating DCs and in doing so contribute to tumor immune evasion [127].

Further supporting a suppressive immunoregulatory role of MCs, it was recently shown that MCs are necessary intermediaries in regulatory T-cell-mediated graft tolerance. In this context, MCs were shown to prevent the rejection of haplo-mismatched skin allografts in tolerant mice. Hence, further investigation is needed to establish whether MCs and their mediators can alter the development of protective T-cell immunity to tumors [128].

6.4 Conclusion

Altogether, the role of MCs during allergic inflammation is very complex, and it is clear that MCs can influence the adaptive immune response by influencing antigen

presentation, as well as T-cell recruitment, activation, skewing and proliferation. It is perhaps easy to see then how these MC-mediated effects in the immune system may as well play an important role in tumor disease. Many studies show that MCs mediate processes that modify the tumor microenvironment in terms of angiogenesis and tissue remodeling that can help tumor grow and metastasize. Furthermore, MCs may also play an influential role in the development of adaptive immunity within the tumor microenvironment. It seems feasible to hypothesize that MCs may act in a tumor-friendly manner through a controlled release of certain mediators while having an anti-tumor effect upon a robust and immediate release of their granular content. As such MCs may play a dual role in propagating the development of cancer. Whether MCs truly regulate the development of anti-tumor immunity, how they do it and the identity of the molecular mediators are currently being studied.

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