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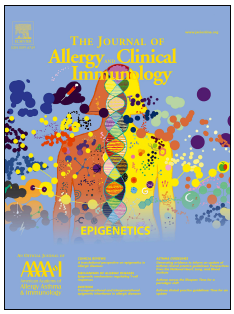

Mast cells as protectors of health

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PII: S0091-6749(18)31605-1

DOI: https://doi.org/10.1016/j.jaci.2018.10.054

Reference: YMAI 13735

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 23 April 2018

Revised Date: 16 August 2018

Accepted Date: 5 October 2018

Please cite this article as: Dudeck A, Köberle M, Goldmann O, Meyer N, Dudeck J, Lemmens S, Rohde M, Roldán NG, Dietze-Schwonberg K, Orinska Z, Medina E, Hendrix S, Metz M, Zenclussen AC, von Stebut E, Biedermann T, Mast cells as protectors of health, *Journal of Allergy and Clinical Immunology* (2018), doi: https://doi.org/10.1016/j.jaci.2018.10.054.

Mast cells as protectors of health

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Disclosure of potential conflict of interest and funding: A. Dudeck has received grants from the Deutsche Forschungsgemeinschaft (DU1172/2-1, DU1172/3-2, DU1172/4-1). S. Lemmens was funded by the 'Agency for Innovation by Science and Technology in Flanders' (IWT-Vlaanderen; 131230). Z. Orinska has received a grant from Deutsche Forschungsgemeinschaft (OR 101/2-1). E. Medina has received grants from the Deutsche Forschungsgemeinschaft (ME 1875/2-1, ME 1875/2-2, ME 1875/5-1). The work of S. Hendrix was supported by DFG grants (SPP1394) and the Fund for Scientific Research Flanders (FWO Vlaanderen; G.0389.12, G0A5813). M. Metz has received grants from Deutsche Forschungsgemeinschaft (ME2668/3-2, ME2668/2-1) and the Else Kröner-Fresenius-Foundation. A. C. Zenclussen was funded by grants from the Deutsche Forschungsgemeinschaft (ZE 526/6-1 and ZE526/6-2) and from the Fritz-Thyssen Stiftung (AZ. 10.08.2.179). E. von Stebut received grants from the Deutsche Forschungsgemeinschaft (STE1208/12-1, STE 1208/13-1, STE1208/14-1, and TR156). T. Biedermann has received grants from Deutsche Forschungsgemeinschaft (BI696/5-1, BI696/5-2, BI696/6-1, BI696/6-2, BI 696/10-1, CRC 685, CRC 824) and Helmholtz Gesellschaft (KKG „Einheit klinische Allergologie") and gave advice to or got a honorarium for talks or research grants from the following companies: Alk-Abelló; Janssen; Meda; Novartis; Phadia Thermo Fisher; Sanofi; Celgene.

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Acknowledgements: Images used in Fig. 1, 2 and 3, *C* were designed by Martin Voss

Abbreviations used

bFGF (Basic fibroblast growth factor), CAF (Cancer-associated fibroblast), CCL (CC chemokine ligand), CMA (Chymase), CNS (Central nervous system), CTMC (Connective tissue-type mast cell), CXCL (CX Chemokine ligand), DC (Dendritic cell), DENV (Dengue virus), DT (Diphtheria toxin), EMT (Epithelial-to-mesenchymal transition, EVT (Extravillous trophoblast), FcεR (Fcε receptor), GM-CSF (Granulocyte-macrophage colony-stimulating factor), IAV (Influenza A Virus), iDTR (Inducible diphtheria toxin receptor), IL (Interleukin), LN (Lymph node), MC (Mast cell), mCMV (Murine cytomegalovirus), MCP (Mast cell proteases), MDSC (myeloid-derived suppressor cell), MHC (Major histocompatibility complex), MMC (Mucosal-type MC), NK cell (Natural killer cell), PEA (Palmitoylethanolamide),RV (Rhinovirus), SA (Spiral arteries), SCI (Spinal cord injury), TAM (Tumor-associated macrophage), TBI (Traumatic brain injury), TGF (Transforming growth factor), TIDC (Tumor-infiltrating dendritic cell), TLR (Toll like receptor), TME (Tumor microenvironment), TNF (Tumor necrosis factor), UmA (Arteria umbilicalis), uMC / uNK cell (Uterine mast cell / natural killer cell), VEGF (Vascular endothelial growth factor), VV (Vaccinia virus), W-sh ($Kit^{W\text{-}sh/W\text{-}sh}$), W-v ($Kit^{W/W\text{-}v}$)

Key words:

mast cell; innate immunity; infection; mast cell protease; tumor; pregnancy; venom; toxin; CNS trauma

Abstract

Mast cells (MC), well known for their effector functions in Th2 skewed allergic and also autoimmune inflammation, become increasingly acknowledged for their role in protection of health. It is now clear that they are also key modulators of immune responses at interface organs like skin or gut. MC can prime tissues for adequate inflammatory responses and cooperate with dendritic cells in T cell activation. They also regulate harmful immune responses in trauma and help to successfully orchestrate pregnancy. This review focusses on the beneficial effects of mast cells on tissue homeostasis and elimination of toxins or venoms. MC can enhance pathogen clearance in many bacterial, viral, and parasite infections, e.g. by TLR2 triggered degranulation, secretion of antimicrobial cathelicidins, recruiting neutrophils or by providing extracellular DNA traps. The role of MC in tumors is more ambiguous, however, encouraging new findings show they can change the tumor microenvironment towards anti-tumor immunity when adequately triggered. Uterine tissue remodeling by α-chymase (MCP-5) is crucial for successful embryo implantation. MCP-4 and the tryptase MCP-6 emerge to be protective in CNS trauma by reducing inflammatory damage and excessive scar formation, thereby protecting axon growth. Last but not least, we see proteases like carboxypeptidase A released by FcεRI activated MC detoxify an increasing number of venoms and endogenous toxins. A better understanding of the plasticity of MC will help to improve these advantageous effects, and hint on ways to cut down detrimental MC actions.

MC orchestrate tissue immunity

Circulating mast cell (MC) precursors migrate to various tissues and mature into multi-facetted effector cells essential for many immune and physiological functions (Fig. 1).

As tissue resident sentinel cells lining interfaces between the organs and the environment, MC critically contribute to the first line of host defense against invading pathogens[1, 2]. MC can recognize and respond to invading pathogens via a wide array of pattern recognition receptors including toll like receptors (TLR), Fc and complement receptors. They can also sense cell stress and tissue damage through a range of receptors including cytokine, alarmin and purinergic receptors[3, 4]. Following activation by Fcε receptor I (FcεRI) or through other stimuli and mediated by a complex machinery including CD63 and other tetraspanins[5], MC undergo degranulation releasing preformed mediators of secretory granules within only minutes followed by the release of a plethora of *de novo* synthesized soluble mediators[3]. Because of this immediate response, MC can respond faster than other tissue resident immune cells to invading pathogens and therefore, in many cases, initiate immune responses.

Mast cell functions have been studied *in vivo* using different models of MC-deficiency or by means of *in vitro* systems of both human and murine MC. However, the informative value of *in vitro* systems is limited since MC act in concert and lively exchange with other tissue resident or immigrating cells and both MC numbers and responses are influenced by the cellular network. Many of the studies addressing the functional relevance of MC *in vivo* have been performed using c-*Kit* mutant mice as models of MC-deficiency. Since the KIT receptor is widely expressed on several subsets of progenitors cells, some findings may account rather for pleiotropic effects of the *Kit* mutation than for specific MC driven effects. For example, studies regarding neutrophil recruitment and functions have to be carefully discussed since beyond MC deficiency, $Kit^{W\text{-}sh/W\text{-}sh}$ (W-sh) mice, which bear the W-sash inversion mutation display neutrophilia[6] while $Kit^{W/W\text{-}v}$ (W-v) mice carrying mutations in the white spotting (W) locus are characterized by neutropenia[7].

In the last years, several groups have developed mouse models that are MC deficient but lack abnormalities related to Kit expression or function (reviewed in[8]). Two strains in which the Cre recombinase is expressed under the MC-specific carboxypeptidase A3 (Cpa) promotor, the $Cpa3^{Cre/+}$ or so-called "Cre-Master" mice[9], and the Cpa3-Cre; $Mcl\text{-}1^{fl/fl}$ or so-called "Hello Kitty" mice[101], are characterized by a constitutive deficiency of all MC subsets and a pronounced reduction in basophil numbers. The Mcpt5-Cre line allows for a constitutive MC depletion when crossed to the $R\text{-}DTA^{fl/fl}$ line in which the diphtheria toxin A chain (DTA) is produced in Cre-expressing cells[11, 12]. When crossed to the iDTR line, in which the diphtheria toxin receptor is expressed by Cre-expressing cells, Mcpt5-Cre iDTR mice can be used for an induced or local MC depletion upon injection of diphtheria toxin (DT)[13]. Of note, due to the expression of the Cre recombinase under the promoter of the MC protease 5, only connective tissue-type MC (CTMC) are depleted in Mcpt5-Cre R-DTA or iDTR mice while mucosal MC and basophil numbers are not reduced[11, 13]. Importantly, the Mcpt5-Cre mice allow for a MC-specific inactivation of certain genes of interest when crossed to the respective floxed line. A further possibility of inducible MC depletion is provided by the "Mas-TRECK" mice in which the DT receptor is expressed under the control of an *Il-4* gene enhancer element[14].

Consequently, comparing previous findings obtained in Kit mutant mice with those obtained with *Kit*-independent mouse models of MC-deficiency or gene inactivation will provide further mechanistic details of MC responses and functions.

MC release preformed mediators and can trigger vascular responses within only minutes after inflammatory insult, in particular vasodilatation and vessel permeabilization resulting in tissue edema[2, 13, 15, 16]. Furthermore, the activation of vessel endothelium and relaxation of connective tissue is a prerequisite for efficient recruitment of neutrophils and T cells to the site of infection/inflammation as well as for the migration of dendritic cells (DC) from the site of infection/inflammation towards the draining lymph nodes (LN), where they will induce antigen-specific immune responses. Indeed, by blocking the activity of MC released histamine on the vasculature, subsequent T cell driven adaptive immune responses are severely impaired[13]. In addition to the vascular effects, MC critically contribute to the initiation of neutrophil recruitment during sepsis and peritonitis[17-20], to sites of skin inflammation[13, 21-23], bone-fracture[24] as well as to areas of atherosclerotic plaque progression[25, 26]. Alongside, MC have been shown to enhance neutrophil effector functions[27, 28]. Mechanistically, release of vasoactive mediators by the MC including tumor necrosis factor (TNF) as well as chemokines and granulocyte-macrophage colony-stimulating factor (GM-CSF) has been demonstrated to be important for neutrophil extravasation and function. MC cooperate with tissue resident macrophages to ensure a fast infiltration of neutrophils and subsequent distribution over the affected tissue[20]. However, the specific connection and communication between MC and macrophages still remains elusive despite their dense network in various tissues. Similar to effects on neutrophils, an impact of MC on DC functionality has been demonstrated under various conditions including bacterial infections, response to pathogen-derived factors and sterile inflammation.

We have shown in various models that upon inflammatory challenges in the skin, MC promote DC migration to skin draining LNs and thereby critically support T cell-driven adaptive immune responses[13, 29]. Consequently, both expansion of $CD4^+$ and $CD8^+$ T cells in the LN and $CD4^+$ and $CD8^+$ T cells homing to affected tissues is markedly reduced in absence of MC. In particular, the peripheral release of TNF by MC has been shown to be required for efficient initiation of skin and airway DC migration to draining LNs[30, 31]. MC-derived TNF predominantly targets $CD8^+$ DC migration and function upon skin inflammation thereby subsequently promoting $CD8^+$ effector T cell-driven immune responses. In addition to effects on DC migration, MC have been shown to promote and shape DC maturation and antigen processing[14, 32-35]. In a very recent study we have shown for the first time *in vivo* that MC and DC undergo a highly dynamic interaction upon skin inflammation which in the further course shifts to long-term synapses. This communication culminates in a protein exchange from DC to MC including MHC class II complexes before DC leave the site of inflammation to migrate to skin draining LNs to prime effector T cells. Surprisingly, the cross-dressing of MC with fully functional active MHC class II complexes by DC equipped MC with antigen-presenting capacity which subsequently enhanced T cell-driven skin inflammation[36].

Consequently, MC initiate and orchestrate innate responses and recruitment of additional innate effector cells as well as promote and regulate adaptive immunity. Here, MC exhibit three modes of action: (1) direct antigen-presenting capacities of MC under certain circumstances, (2) the modulation of DC migration and effector T cell priming efficiency, (3) the recruitment of effector T cell subsets to sites of inflammation or infection and the on-site activation of homing T cells to drive efficient inflammatory responses[36, 37]. Collectively, MC represent sessile tissue sentinels that highly communicate with neighboring tissue resident DC and macrophages, initiate vascular responses and orchestrate the recruitment of

additional innate and adaptive effector cells and their subsequent activation to ensure effective immune responses and restore tissue and barrier integrity (Fig 2). The appreciation of MC function is clouded by their adverse effects in allergy and anaphylaxis. But by releasing high quantities of a broad variety of pro-inflammatory mediators, MC critically contribute to acute host defense to invading pathogens.

The multifaceted roles of MC in host defense against infection

MC have been well documented to exert a protective role in the host defense against **bacterial infections**[1, 2, 38]. Detection of invading bacteria is afforded by an array of receptors including TLR, FimH receptor (CD48) and complement and Fc receptors. Sensing of bacterial products and humoral factors of the innate immune system results in MC degranulation with the concomitant release of a wide selection of biological active antimicrobial compounds and pro-inflammatory cytokines and chemokines[39-42]. Consequently, MC critically contribute to the host defense against invading bacteria by two modes of action: (1) direct antimicrobial effect and (2) the recruitment and activation of inflammatory cells to the site of infection. For example, it has been reported that MC-mediated release of TNF promotes neutrophil recruitment to the site of *Klebsiella pneumonia* infection[18], while secretion of cathelicidins by MC has direct bactericidal effects on *Streptococcus pyogenes*[43]. In this context, it has been shown that MC undergo degranulation upon TLR2-mediated sensing of *Staphylococcus aureus*[44] and *Enterococcus faecalis*[39], resulting in the release of granule mediators that are very efficient at inhibiting pathogen growth. Furthermore, *S. aureus* δ-toxin induced MC degranulation may be a major mechanism by which Th2 skin inflammation is exacerbated in atopic lesions[45]. MC can also directly participate in bacterial killing through phagocytosis[46] or by the release of extracellular traps (Fig 3, *A*), composed of DNA, histones and MC-specific granule proteins like tryptase and CRAMP/LL-37 where pathogens are captured and killed[39, 44, 47-49].

However, some pathogens have evolved sophisticated strategies to counteract the antimicrobial activities of MC[44, 48, 50, 51]. In this regard, it has been reported that *Escherichia coli* can evade MC phagocytic killing by entering into a compartment within the MC that bypasses phagolysosomal fusion and facilitates bacterial survival[51]. This process was mediated by engagement of CD48 on MC by the bacterial mannose-binding moiety FimH of type 1 fimbriae[51]. *S. aureus* evades the extracellular killing activity of MC by promoting its internalization within these cells into a niche that is permissive for bacterial survival[44, 48]. In contrast to type-1 fibriated *E.coli, S. aureus* alpha-hemolysin mediates internalization within MC by a mechanism that involves fibronectin, forming a bridge between fibronectin-binding proteins expressed on the bacterial surface and the α5β1 integrin expressed on the surface of the MC[44, 48]. Because *S. aureus* can survive for long-terms within MC[44, 48], it is conceivable that MC may serve as a reservoir of viable bacteria, thus providing another explanation for the high rate of skin infections / erysipelas and cellulitis associated with atopic dermatitis[52, 53], a chronic inflammatory skin disease associated with high amount of MC in the affected lesions[54].

MC also play a role in **viral infections** and can detect infecting viruses either directly or indirectly by sensing of danger signals released from infected cells (alarmins) and of

mediators produced in the context of the antiviral response (cytokines, interferons). Depending on the specific sensing pathway, MC respond by degranulation, release of lipid mediators or production of cytokines/chemokines. Beyond direct antiviral effects, MC support the antiviral host defence by recruitment and conditioning of additional effector cells, in this case natural killer (NK) cells[55, 56], NK-T cells[57, 58] and $CD8^+$ T cells[59-61].

Mechanisms of MC antiviral response have been studied in different experimental infection models in mouse and human cell culture systems. MC have been reported to be direct targets of murine cytomegalovirus (mCMV)[62], Vaccinia virus (VV)[63], and Dengue virus (DENV)[64-66], resulting in degranulation and robust cytokine and chemokine response. MC activation by mCMV results in two waves of degranulation: a rapid, early MC degranulation requiring TLR3/TRIF signalling in neighboring non-MC, and a delayed, TLR3/TRIF-independent degranulation – most likely in response to viral replication[67, 68]. Importantly, MC promote the recruitment of protective short-lived effector $CD8^+$ cells in a CC chemokine ligand (CCL)5-dependent mechanism[61, 62] and the reduced lung infiltration by $CD8^+$ T cells in MC-deficient mice has been demonstrated to be associated with a more severe mCMV infection. In contrast, during VV skin infection, MC degranulation is induced through the interaction of sphingosine-1-phosphate receptor 2 (S1PR2) with viral membrane lipids[63]. Similar to bacterial infections, the MC degranulation exerts direct antiviral effects by the release of cathelicidin thereby inactivating VV and decreasing the viral load. Studies of DENV infections in MC-deficient mice revealed that the recruitment of NK- and NKT-cells was reduced in absence of MC in contrast to an enhanced number of tissue macrophages[57, 69]. Localized MC responses to DENV therefore seem to be protective through the recruitment of different immune cells and viral clearance. However, upon degranulation of infected skin MC, the virus could be detected within MC granules that were subsequently transported to skin draining lymph nodes, a process that may contribute to the systemic spread of DENV infection from the initial site of virus invasion[66]. Moreover, the systemic MC activation and release of VEGF and MC proteases (MCP) may account for generalized vascular effects including the increase of vascular permeability resulting in severe Dengue hemorrhagic fever, and Dengue shock syndrome[70]. Hence, inhibition of MC degranulation induces improvement of clinical symptoms[71]. Since DENV specific IgE titers were increased in patients suffering from Dengue hemorrhagic fever or shock syndrome[72], FcεRI-mediated MC activation could result in an increased MC reactivity in line with observed elevated interleukin (IL)-9 and IL-17 levels[70]. Being sentinel cells in human lungs, MC get in contact with human respiratory pathogens like Influenza A Virus (IAV) and Rhinoviruses (RV). They probably shape lung-specific immune reactions and are involved in early stages of antiviral response in concert with airway epithelial cells, alveolar macrophages and DC. Here, MC-related effects could be both – protective and detrimental. Upon infection by IAV *in vitro*, cytokine and chemokine production by MC depends on cytoplasmic RNA-sensor retinoic acid-inducible gene I (RIG-I)[73], potentially contributing to the excessive host immune reaction against the IAV. Consistently, W-sh mice were resistant to IAV-induced inflammatory disease[73]. Lung gene expression indicated stronger MC recruitment in 2009 H1N1 MA-CA/04 infected BALB/c mice compared with the less virulent prototypic 2009 H1N1 CA/04 strain[74] and MC progenitors were recruited to lungs of mice intranasally infected with H1N1 Influenza A/PR8 virus[75]. On the other hand, MC responses to IAV might be strain specific and sometimes also limiting inflammation caused by less pathogenic strains[76]. IAV infection of bone marrow derived mast cells *in vitro* leads to MC degranulation[8, 77] in line with the described increase of histamine levels in nasal mucosa upon IAV infection in mouse[78]. It remains unclear whether local MC activation in context of IAV infection is essential for the establishment of a stable, long-lived memory T cell pool and specific antibody production. RV

infection is strongly associated with asthma exacerbations[79] and the induction of histamine release and IL-8 or GM-CSF production were initial observations regarding the RV-induced MC response[80]. Since allergic sensitization modifies the phenotype of rhinovirus infections, blocking IgE by the anti-IgE monoclonal antibody Omalizumab decreased susceptibility to RV infections, reduced viral illness and viral shedding duration and peak[81, 82]. It should be further evaluated whether FcεRI-mediated signals directly inhibit antiviral MC response and whether Omalizumab treatment affects MC antiviral response.

The role of MC in **parasite infections** remains controversial and seems to depend on the specific parasite species and site of infection. Host defenses against helminth infections are mediated by the activation of Th2 cells, ILC_2, eosinophils and MC in line with increased levels of cytokines such as IL-4, IL-5 and IL-13[83-86]. MC have been reported to exert direct cytotoxic effects on helminths via secretion of serine proteases (chymase and tryptase)[42, 85, 87]. Furthermore, MCP-1 has been shown to increase the intestinal epithelial barrier permeability resulting in increased luminal flow and thereby in parasite expulsion[85]. Adaptive immune responses against helminths are further modulated by MC via soluble mediators or cell-cell interactions with DC and other antigen-presenting cells[42]. For example, infections with *Strongyloides ratti*[88-90], *Trichinella spiralis,* and *Nippostrongylus brasiliensis* are associated with high numbers of infiltrating MC to sites of infection, implicating the important role of MC in host defense[42, 83, 85, 88]. Human MC_T have been shown crucial for the expulsion of nematodes [cite: Huber et. al. Regulation of the pleiotropic effects of tissue resident mast cells, in this issue] It has also been shown in a *T. spiralis* infection model, that proteases expressed by infection induced mucosal MC (MMC) varies with the type of infected tissue[91].

MC activation and degranulation seem to play a pivotal role in parasitic protozoan diseases. Infections with *Plasmodium spp*., *Trypanosoma spp*., and *Toxoplasma gondii* are associated with MC accumulation and increased MC degranulation both in humans and mice[88]. Furthermore, studies in mice infected with *Trypanosoma spp*. or *T. gondii* revealed a higher parasite burden and increased lethality in absence of MC accompanied by lower levels on TNF and IFN-γ, respectively[88]. In leishmaniasis, skin MC have been demonstrated as a niche for the intracellular parasite (Fig 3, *B*) and *Leishmania major* infection leads to MC degranulation and release of pre-formed TNF within only minutes[92-94]. Interestingly, it has been recently shown that MC/parasite interaction also resulted in ROS production and formation of extracellular traps leading to parasite killing[95].

Defense mechanisms against intracellular pathogens (e.g. mycobacteria, *Leishmania*) are characterized by granuloma formation to prevent dissemination. This process is initiated by neutrophil recruitment, followed by invasion of macrophages and formation of a T cell wall. Importantly, MC[96] and in particular, MC-derived TNF are prerequisite for neutrophil recruitment towards the site of parasite encounter, which in turn induces macrophage immigration via MIP-1α/β. Along this line, the impaired neutrophil and macrophage recruitment to sites of infection in MC-deficient mice was associated with enhanced parasite spreading from skin to spleen[94].

Parasite elimination and healing in murine cutaneous leishmaniasis, however, critically relies on adaptive responses, in particular the induction of IFNγ producing Th1/Tc1 cells[97]. IFNγ subsequently mediates the activation of infected macrophages to produce NO, which ultimately eliminates the parasites. Despite the rapid MC degranulation and their impact on neutrophil and macrophage recruitment, the role of MC for disease outcome is still not fully

understood. In the mouse model, in absence of MC, *L. major* inoculation leads to larger lesions, higher lesional parasite burdens, and enhanced visceralization associated with predominant Th2 immune responses or impaired induction of both Th1 and Th17 cells, respectively[94, 98]. Local reconstitution of MC-deficient mice with MC did abrogate the effect. This may be explained by the direct cross-talk between MC and DC resulting in DC maturation and preferential priming of Th1 cells and Th17 cells[35]. In addition to modulating DC maturation and granuloma formation, MC-derived IL-4 or TNF may contribute to this effect, since these cytokines have been shown to directly promote Th1 development[99]. In contrast, Paul *et al.* recently reported that MC-deficient mice independent of *Kit* mutations did not exhibit an altered progress of *L. major* infection with regard to lesion sizes, parasite burdens or cytokine responses compared to wild type BALB/c or C57BL/6 mice, albeit effects on inflammatory cell recruitment were not studied[100]. Information on the role of MC in infected patients is not available.

In conclusion, the ample variety of MC mediators allows for multifaceted effects promoting host defences against bacterial, viral or parasite infection. On one hand, MC exert direct antimicrobial or cytotoxic effects in particular via the release of proteases, antimicrobial agents as cathelicidin, ROS and extracellular traps. On the other hand, MC efficiently initiate the recruitment of additional innate and/or adaptive effector cells, i. e. (dependent on the type of response) neutrophils, monocytes/macrophages, NK cells and NKT cells, or eosinophils. And finally, MC critically enhance the induction of adaptive responses towards the infection by directly promoting T cell activation or by modulating the migration and functionality of DC. A better understanding of MC-mediated effects on innate responses and the induction and regulation of adaptive immunity in the context of host defense against bacterial, viral or parasite encounter will therefore unveil new immunotherapeutic intervention strategies in order to generate immune protection, resolve inflammation and limit tissue damage (Fig 3, *C*).

A protective role of mast cells in the context of tumor development and progress

Inflammation not only activates immune defenses against pathogens but also triggers cellular events that are involved in tumor development, progression or its defense. Aberrant immune signals can promote malignant transformation of cells and carcinogenesis. Several inflammatory mediators, such as TNF-α, transforming growth factor (TGF)-β or IL-10 have been shown to contribute to both initiation and progression of cancer and MC have been shown to be major contributors to their release[101]. Chronic inflammation is a key feature of the tumor microenvironment (TME), not only stimulating proliferation and survival of tumor cells but also suppressing anti-tumor immunity. Among the cells that contribute to the effects of immune suppression within the TME are tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), tumor-infiltrating dendritic cells (TIDC), and cancer-associated fibroblasts (CAF)[102-104]. Recently, MC have been increasingly acknowledged as potential players of relevance within the TME as they are long-lived[105], frequently detected in the TME[106], and characterized by functional plasticity[107, 108]. Accumulation of MC in the TME was reported especially in melanomas[109]. MC are important immune sentinels with the ability to enhance T cell-mediated immune reactions and were shown to drive immune responses under other circumstances[13, 22]. With their functional plasticity depending on the specific TME, the existing debate whether MC promote tumor growth and metastasis or drive immune surveillance and tumor clearance is likely a question of orchestration rather than

determination[110]. Increasing knowledge on MC in the context of cancer will enable us to translationally target MC and their products in the future. Furthermore, c-Kit mutation independent mouse models will provide a more reliable functional understanding to complement observations in patients. Examples of how MC influence tumor behavior are given below.

MC can be recruited and activated by factors like SCF or IL-3 that may also be provided by tumors. Notably, MC recruitment to tumors may be independent from tumor infiltration by other immune cells in mice and humans alike[111, 112], and in case of c-*Myc* oncogene driven β-cell tumors, mouse MC recruitment via CCL5 could be directly related to MYC activity and thus to oncogenic transformation[113]. It has also been shown that pro-angiogenic and proliferation promoting MC factors like basic fibroblast growth factor (bFGF), IL-8 or TGF-β can enhance tumor vascularization and growth and MC are a major source of vascular endothelial growth factor (VEGF). IL-8 secretion by activated MC has been furthermore shown to induce epithelial-to-mesenchymal transition (EMT) of thyroid cancer cells, thereby promoting tumor invasiveness in a mouse xenograft model[114]. In the process of EMT, cells de-differentiate and de-polarize, loosing epithelial and gaining stem cell properties. It enables tumor cell migration and contact independent growth and thus the formation of tumor metastases. How MC orchestrate EMT is still a matter of debate, but association of MC with melanoma de-differentiation in mice has been shown[115]. Accordingly, in invasive melanoma, higher MC numbers have been found than in melanoma in situ, which in turn had higher MC numbers than benign melanocytic nevi[109]. In one study, W-v mice showed reduced growth of B16 melanomas compared to control mice based on inhibited vascularization indicating a crucial role for MC in melanoma-associated angiogenesis[116]. MC numbers and degranulation also correlated with progression of primary cutaneous lymphoma. In line with this finding, MC supernatant induced pro-inflammatory cytokine release and proliferation of primary cutaneous lymphoma cells *in vitro*, while growth of a lymphoma cell line *in vivo* as well as tumor vascularization was decreased in mice lacking CTMC (*Mcp5-Cre*$^{+}$*/iDTR*$^{+}$)[117]. In addition, it was described that destruction of tissue integrity and degradation of the extracellular matrix by MCP supports tumor spread and that MC can suppress anti-tumor immunity by IL-10 secretion and IL-10 induction by histamine[112, 118].

Despite this evidence, in recent years, a role of MC anti-tumor activity has increasingly been appreciated. This has been stimulated to some extend by the propagation of the “master switch” hypothesis by Melissa Brown and others[119], describing MC as local immune supervisors. MC have been found beneficial for the rejection of some tumors sensitive to TNF-α[120, 121]. In addition, MCP apparently not only promote tumor spread but also anti-tumor effects in melanoma. Mice deficient for multiple MCP showed reduced numbers of cells expressing MHCI like protein CD1d that mediates antigen presentation to invariant chain natural killer T (NKT) cells and lower levels of the T cell and NKT cell recruiting CX chemokine ligand (CXCL)16 in lungs bearing higher numbers of B16F10 melanoma metastases[122]. Consistently, IL-9 secreting T cells could mount robust B16F10 immunity dependent on MC[123]. Further anti-tumor effects of MC include induction of tumor cell apoptosis or eosinophil recruitment by MCP and IL-5[124]. A study at Lund University found high MC density to be associated with improved prognosis in colon cancer patients[125]. In line with this finding, in a mouse model of circulating colon cancer metastases, anti-tumor vaccination proved to be effective via Th9 cell and MC activation[126], pointing out a possible way how MC could potentially be used as a target in anti-tumor therapy. Because MC are recruited early and in considerable numbers to many tumors, and because of their plasticity, they are essential players in a number of novel therapeutic strategies aimed on solid tumors[127].

Thus, tumor biology and behavior orchestrate phenotype and function of MC. They demonstrate MC plasticity and also a 'personalized' role of MC in regard to specific behavior and overall effects on tumor progression. Based on the accumulation of MC at tumor sites and their fundamental plasticity, MC may be ideal additional targets within the TME to further enhance tumor immunotherapy in the future. Immune checkpoint inhibitors, releasing the brakes on tumor infiltrating effector T cells prolong overall survival in cancer patients and have been a major advance in the therapy of melanoma and other tumors[128]. These therapies aim to correct the functioning of tumor specific T cells setting up tumor immune defense. However, other cells within the TME may also be targets of intervention, among them MC. Modulation of MC behavior might be used to further diminish T cell inhibition by the TME, to recruit additional T cells, and even to directly target the tumor. To this end, understanding how MC influence tumor development and subsequent fate is pivotal for the decision to either interfere with or to augment MC function. Thus, some MC functions could be inhibited, whereas others contributing to tumor immune clearance could be enhanced, e.g. by activating MC with danger signals such as TLR ligands. For example, TLR3 ligand poly(I:C) has been demonstrated to trigger $CD8^+$ T cell recruitment by MC release of interferon-β, CXCL10 and other attractants[129] and to be effective as a component of anti-tumor vaccination in mice[126]. Another potential therapeutic option, using tumor specific IgE as a tool for MC activation, is encouraged by the inverse correlation of allergy and atopy with some tumors[120, 127] and the protective role of IgE induced by carcinogen induced tissue damage (although current evidence points at basophils)[130]. However, more evidence is needed to establish MC-based therapeutic approaches in cancer immunotherapy.

Uterine mast cells and natural killer cells skew feto-maternal immune cross-talk towards fetus tolerance

While novel anti-tumor strategies aim at eliminating the ability to induce tolerance or angiogenesis from the diverse repertoire of MC actions, these are key functions that make MC guardians of fetal implantation. MC populate the reproductive tract[131, 132], they cyclically expand and are activated by hormones[133, 134]. They are abundant in uterus and placenta as we could confirm by in vivo 2-photon microscopy[135]. Histamine produced and released by MC is reportedly involved in blastocyst implantation[136]; however, histamine production can be triggered in MC-deficient mice by steroids[137], suggesting other sources than MC. This may also explain why implantation is impaired but not totally abolished in W-sh mice[138]. Accordingly, histamine receptor blockers negatively affect fertility by hindering ovulation[139] and implantation[140, 141] in experimental models. No evidences exist for patients on chronic antihistamines regarding their ability to get and stay pregnant.

Uterine MC (uMC) represent a distinct population composed of both MMC as well as CTMC[142] and a third, intermediate MC population[134]. These cells, already described for other tissues, reportedly reflect different stages of differentiation[143, 144] or are undergoing a transdifferentiation process, changing their content in proteoglycans, amines and peptides depending on the environment[145]. This points out the uniqueness of uMC that are characterized by a high plasticity much needed for the different stages of pregnancy.

One of the most relevant pregnancy milestones is the remodeling of the spiral arteries (SAs), a pivotal adaptation to gestation[146]. Inadequate vascular changes and impaired SA remodeling can cause preeclampsia, intrauterine growth retardation (IUGR), preterm birth or miscarriage[147-149]. It was long believed that uterine NK cells are the only innate immune cells

relevant for remodeling. However, their absence or depletion did not profoundly affect pregnancy[150, 151]. Our recent works revealed that uMC play an unsuspected, pivotal role for remodeling and fetal survival. Animals devoid of MC had abnormally remodeled SAs and presented IUGR. This was true for both W-sh and *Kit* mutation independent MC deficient *Cpa3-Cre* mice[138, 151]. Interestingly, combined absence of NKs and MC worsened the IUGR phenotype, with more than half of the fetuses growth-retarded[151].

Mast cell proteases such as chymase, tryptase and carboxypeptidase A, account for the largest proportion of the protein content in secretory MC granules, and they can also be released from MC within seconds after activation. Although many potential targets of MCP have been identified *in vitro*, their *in vivo* relevance of had long been ill understood. They are involved in a number of pathologies (like arthritis and allergic airway inflammation), but also have been shown to be protective against infecting pathogens[152]. Furthermore, MCP are in involved in tissue remodeling in tumor and pregnancy, as well as in trauma and detoxification as will be described later in this chapter.

We detected α-chymase (MCP-5) in MC but also in uterine NK cells in mice[153]. *Mcp5* gene expression by a fraction of uNKs was confirmed in MC deficient *Cpa3-Cre*. In wild type mice, it cannot be excluded that uNKs may acquire MCP-5 after the interaction with uMC, but unlike the interaction between DC and MC[36], the transfer of cytoplasmatic material from MC to NK cells still needs to be investigated. Moreover, uNKs and uMC seem to counterbalance each other in order to ensure SA remodeling[154]. We showed that MCP-5 mediated apoptosis of uterine smooth muscle cells *in vitro*, a key feature of SA remodeling. Mice with selective deletion of MCP-5$^+$ cells had un-remodeled SAs and growth-restricted progeny[153]. Further research is need to analyze the role of MCP-5 in human reproduction. De Leo et al. (2017) described the existence of three human subtypes of uMC. They express hormone receptors, suggesting that their function is altered by local hormones[155]. We confirmed the existence of MC at the feto-maternal interface in first trimester human pregnancy and revealed their close proximity to invading trophoblasts (EVTs). Interestingly, fluorescence images showed that MC expressing the human α-chymase (CMA-1) might have interacted with trophoblasts, maybe forming a similar synapse as with DC. MC supernatant but also human recombinant CMA-1 stimulated *ex vivo* migration of human trophoblasts, a pre-requisite for SA remodeling[154]. Thus, chymases secreted by uMC/uNKs are pivotal to the vascular changes required to support pregnancy. The magnitude and importance of this phenomenon was recently studied *in vivo* by following up mouse pregnancy and fetal development by high frequency ultrasound. The combined absence of uMC/uNKs negatively impacted pregnancy from mid gestation onwards leading to smaller implantation sizes and reduced placental dimensions that were further associated with absent or reversed end diastolic flow in the *arteria umbilicalis* (UmAs) of some fetuses of uNK/uMC-deficient mice but not of wild type mice[151]. Moreover, mice that were spontaneously prone to abortions had insufficient numbers of uMC. The adoptive transfer of Treg cells specific to paternal antigens, an established therapy to restore pregnancy, normalized the number of uMC and in turn positively influenced the remodeling of spiral arteries and placenta development, normalizing sFlt-1 levels[156]. Hence, we speculate that in addition to their interactions with uNKs and trophoblasts, uMC team up with Treg to promote pregnancy. Whether this occurs via direct cellular interaction or through released mediators needs to be studied in more detail.

The adaptability of uMC to the environment is highlighted by recent data on increased systemic infection leading to pre-term birth in *Mcp4*-deficient mice[157]. This indicates that in

pregnancy, MC not only act as relevant actors in implantation and uterine remodeling, but can also overtake an important role in defending mother and fetus against infections.
Overall, MC emerge as essential modulators of the immune response during pregnancy. They exert different roles, mediating implantation, angiogenesis and fostering fetal-tolerance but retain their abilities in pathogen defense if mother or fetus are in danger (Fig. 4).

Mast cells and MCP-4/6 in CNS trauma

MC are typically located close to outer layers and barriers, such as epithelial borders, mucosal membranes, and vascular walls because they are the first line of defense against invading pathogens, environmental antigens and allergens, or environmentally derived toxins. In the healthy central nervous system (CNS), MC are typically found in the meninges, choroid plexus, olfactory bulb, mesencephalon, and the parenchyma of the thalamic-hypothalamic region. They generally reside alongside the blood vessels. In CNS disease context, MC were detected in brain infarcts and at the edge of multiple sclerosis (MS) plaques (reviewed in[158]).
MC may exert either beneficial or detrimental or no effects in different CNS diseases such as multiple sclerosis, stroke and Alzheimer's disease - depending on the models and methods used[158-162].
Similarly, there are contradictory findings on the role of MC during and after CNS trauma such as traumatic brain injury (TBI) and spinal cord injury (SCI)[158, 161, 162]. After TBI, MC numbers increase for weeks and contribute to the brain damage by releasing inflammatory mediators such as TNF and IL-9. Inhibition of MC activation decreased the brain damage in the immature rat brain indicating detrimental MC effects[163, 164]. On the other hand, palmitoylethanolamide (PEA) decreased MC numbers in the brain of experimental TBI mice, inducing beneficial effects on edema, infarct volume and behavioral effects[162].
Our own data indicate a protective role of MC after TBI[165] and SCI[166, 167]. In the context of TBI, we have shown that MC-deficient W-v and W-sh mice display increased neurodegeneration in the lesion area after brain trauma[165]. Furthermore, MC-deficient mice displayed an increased presence of macrophages/microglia, as well as dramatically increased T-cell infiltration, combined with increased astrogliosis. The number of proliferating $Ki67^{+}$ macrophages/microglia and astrocytes around the lesion area was also highly increased compared to wild type mice. We further analyzed whether the role of the MC-specific chymase MCP-4 in our SCI model. Mice deficient in MCP-4 revealed that astrogliosis and T-cell infiltration were significantly increased. Treatment with an inhibitor of MCP-4 significantly increased macrophage/microglia numbers and astrogliosis. These findings suggest that MC exert protective functions after brain trauma, at least in part, via MCP-4.
Consistently, MC display protective functions after SCI. W-sh mice displayed significantly increased astrogliosis and T cell infiltration as well as significantly reduced functional recovery compared to wild type mice[166]. In addition, W-sh mice show significantly increased protein levels of MCP-1, TNF-α, IL-10 and IL-13 in the spinal cord. Mice deficient in MCP-4 also showed increased MCP-1 and IL-13 levels, along with more IL-6 in spinal cord samples and a decreased functional outcome after spinal cord injury. In line with these findings, a degradation assay using supernatant from MC derived from either $MCP\text{-}4^{-/-}$ mice or controls revealed that MCP-4 cleaves MCP-1, IL-6 and IL-13 suggesting a protective role for MCP in neuro-inflammation. These results indicate that MC may reduce CNS damage by degrading inflammation-associated cytokines via MCP-4.

Since MCP-4 is also involved in tissue remodeling and extracellular matrix degradation we have further investigated whether MC modulate the glial and fibrotic scar after SCI[168]. We have shown that the decrease in locomotor performance in MCP-4$^{-/-}$ mice is associated with an increased lesion size and excessive scar formation[168]. The expression of axon-growth inhibitory chondroitin sulfate proteoglycans was dramatically increased in the perilesional area in MCP-4$^{-/-}$ mice compared to wild type mice. Moreover, the fibronectin-, laminin-, and collagen IV-positive scar was significantly enlarged in MCP-4$^{-/-}$ mice at the lesion center. *In vitro* MCP-4 directly cleaved collagen IV. On the transcriptional level, neurocan and GFAP were up-regulated in the MCP-4$^{-/-}$ group at day 2 and day 28 after injury, respectively. Our data showed that MCP-4 modulates scar development after SCI by changing the gene and protein expression patterns of key scar factors *in* vivo thereby suggesting a new mechanism via which mMCP-4 may improve recovery after SCI.

We further investigated the protective effects of MCP-6, a MC-specific tryptase[167]. Functional recovery was significantly impaired in MCP-6$^{-/-}$ mice after SCI. This decrease in locomotor performance was associated with an increased lesion size and excessive scarring at the injury site. Axon growth–inhibitory chondroitin sulfate proteoglycans and the extracellular matrix components fibronectin, laminin, and collagen IV were significantly up-regulated in the MCP-6$^{-/-}$ mice. MCP-6 directly cleaved fibronectin and collagen IV *in vitro*. In addition, gene expression levels of the scar components fibronectin, aggrecan, and collagen IV were increased in MCP-6$^{-/-}$ mice in the subacute phase after injury. These data indicate that MCP-6 has scar suppressing properties after SCI via indirect cleavage of axon growth–inhibitory scar components and alteration of the gene expression profile of these factors.

These findings are consistent with studies in fibrotic conditions outside the CNS where a profound accumulation of MC has been described. The effects of MC and their secreted factors on fibrosis are divers depending on the model and the phase of the injury or disease. For example, tryptase is involved in ECM degradation, whereas CCL2 induces fibroblast proliferation and chemotaxis. An extensive overview of the different secreted mediators and their involvement in fibrosis is provided by Bradding and Pejler[169]. Both, pro-fibrotic and anti-fibrotic roles for MC have been described. It has been postulated that *acute* inflammatory stimuli lead to anti-fibrotic activity whereas *chronic* or *repeated* stimuli lead to pro-fibrosis. Our murine models of CNS trauma represent the highly acute to early chronic phases of CNS damage and repair. Hence, we would expect an anti-fibrotic activity. Consistently, we see an increase of scar components in our MC knockout mouse models similar to anti-fibrotic effects of mast cells characteristic for acute rodent models of fibrosis. This is in contrast to human fibrotic diseases which often progress over many years and are associate with pro-fibrotic activities of MC[169]. However, it is important to note that MC research after CNS injury is still in its infancy and no human studies are available yet. Rodent models of spinal cord and brain injury display substantial differences compared to the human situation. Three points are of particular importance when analyzing MC effects in the CNS: The immune system of mice after CNS injury is biased toward T cell responses while humans show a much higher impact of humoral immunity. Thus, MC effects on CNS inflammation may differ substantially between humans and rodents. Secondly, rodents display a surprisingly fast spontaneous recovery after incomplete SCI (only full transection of the spinal cord leads to chronic paralysis). Thus, rodent models have important limitations to represent the human clinical situation which is characterized by an absence of substantial spontaneous recovery. Thirdly, MC reconstitution is a gold standard technique to distinguish between MC-dependent and independent effects in the CNS. Unfortunately, in rodent MC models MC reconstitution in the CNS is incomplete (review in[158]). Therefore, the investigation of specific MC proteases

such as mMCP-4 and mMCP-6 may be more instructive to further analyze MC functions after CNS injury.

In conclusion, MC exert protective effects after CNS trauma in mice via MCP-4 and MCP-6, leading to functional improvement after injury. Both proteases modulate gene expression and induce cleavage of selected scar components which inhibit axon growth. In addition, MCP4 acts anti-inflammatory by degrading inflammation-associated cytokines which contributes to the reduction of CNS damage and, hence, improved functional recovery.
An important open question is whether MC play specific and antagonistic roles in different phases of traumatic and chronic neurodegenerative diseases. It is tempting to speculate that MC may exert pro-inflammatory functions during highly acute injury processes, protective, anti-inflammatory and anti-fibrotic functions during *early* chronic remodeling, and pro-fibrotic effects in *later* chronic phases. However, systematic studies have yet to be performed to address this hypothesis of phase-specific MC effects in CNS pathologies.

Role of mast cell proteases in homeostasis and protection against endogenous toxins

Many studies in mice have provided evidence that MC have the ability to protect against bacterial infection, for example by releasing TNF and other pro-inflammatory mediators, but also by other mechanisms including the release of proteases (reviewed in detail in[2]). In severe bacterial infections such as sepsis, endogenous peptides are produced that can be detrimental to the host. In mouse models of septic shock, it has been shown that MC proteases can promote homeostasis by degrading for example endothelin-1 and neurotensin-1, and thus inactivate and "detoxify" these peptides[170, 171]. Similarly, MC proteases have been shown to effectively degrade alarmins, such as heat shock protein 70 and IL-33, resulting in the control of the potentially harmful inflammation associated with an increased concentration of these substances in tissues[172]. Human MC tryptase efficiently degraded snake venoms *in vitro*[173] and epidemiological evidence suggests that previous sensitization is critical for MC mediator release of venom exposed patients[174]. In combination, these findings suggest that a hypersensitivity reaction might be an effective mechanism providing protection from venoms and toxins.

Role of mast cell proteases in the protection against animal venoms

MC-derived proteases can promote homeostasis through the limitation of endothelin-1-induced toxicity. These findings lead to investigations on similar detoxifying abilities of MC in response to the venom of the Israeli mole viper (*Atractaspis engaddensis*), as the amino acid sequence of endothelin-1 has a high similarity to that of sarafotoxin 6b, the most toxic component of the snake's venom. Indeed, MC-derived carboxypeptidase A was found to be able to degrade and thus detoxify the venom and lead to an enhanced protection against its toxic effects in mice [175, 176]. Moreover, in subsequent studies, numerous different phylogenetically distinct animal venoms have been found to activate MC and to be strongly reduced in their toxicity by proteases released from MC[175, 177] (Table1).

Apart from their above described beneficial functions, MC are generally known for their important role as effector cells in allergic responses. Here, MC are activated by specific IgE antibodies that can be produced against any of a broad range of seemingly harmless

antigens,[178] but also to venom components. It has therefore been speculated that the IgE-mediated strong activation of MC by venom-specific IgE antibodies can actually contribute to an enhanced resistance against the toxicity of the venoms. This hypothesis has already been put forward by Margie Profet in 1991, who proposed the "toxin hypothesis of allergy", in which she postulated that acute allergic reactions evolved as a defense mechanism, allowing the sensitized host to respond promptly to, and to expel, neutralize and/or avoid, noxious substances which might be indicative of potentially life-threatening situations[179, 180]. However, sublethal toxin doses, e.g. of *Hymenoptera* venoms frequently provoke severe immune reactions as well, some resulting in life threatening anaphylactic reactions rather than being protective.

Using mouse models of active sensitization to either bee or viper venom, it has been shown that the production of venom-specific IgE antibodies can indeed limit the toxicity of the respective venoms[181, 182]. Both systemic or local anaphylactic responses to the venoms lead to an IgE- and FcεRI-dependent activation of mast cells and a subsequent enhanced likelihood to survive a challenge with a potentially lethal dose of the venom[181, 182], indicating that an allergic activation of MC can indeed protect the host against noxious substances.

Thus, mast cell proteases are important enzymes involved in maintaining tissue homeostasis and in protecting the host from potentially dangerous substances.

The ability of MC to immediately release proteases upon contact with potential toxins can be regarded as one of the crucial physiological functions of MC. Some venom constituents, including mastoparan, which has recently been shown to activate MC via the MRGPRX2 receptor, can degranulate MC independent of prior sensitization[183] and local tissue edema, that limit venom absorption require MC but no sensitization as well[184]. Furthermore, recent evidence showing that the production of IgE against venom components can enhance survival after subsequent venom exposure indicates that the development of an allergy to venom components, but also to other potentially dangerous substances, should not only be considered as a misguided Th2 response leading to potentially lethal anaphylaxis, but also to a physiological function leading to an enhanced protection against environmental threats.

Conclusion and outlook

Recent work has significantly increased our knowledge of MC contribution to immune reactions in a variety of conditions and their role at the interface between environment and the host has been understood much better. The classic view of MC as main contributors to allergic inflammation, another function on interface organs, has to be complemented because MC emerge as multi-faceted immune modulators and operators of health at interfaces (Fig. 1). Future research therefore will need to ask i) what is the beneficial advantage of a MC behavior in a given situation? ii) what are the drivers and modulators that determine MC behavior and function? and iii) how could one direct MC action towards an advantageous outcome? This review highlighted some of the most recent advances supporting this new view on MC function, but more research is needed to be able to specifically target MC for exerting a role as protector of health.

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Tables

Order	Species	Common name
Serpentes (snakes)	*Atractaspis engaddensis*	Israeli mole viper
	Crotalus atrox	Western diamondback rattlesnake
	Agkistrodon contortrix contortrix	Southern copperhead
	Echis carinatus	Saw-scaled viper,
	Bothrops atrox	Common lancehead
	Daboia russelii	Russell's viper
	Naja pallida	Red spitting cobra
Arachnida (spider)	*Loxosceles reclusa*	Brown recluse
Hymenoptera	*Apis mellifera*	European honey bee
Scorpiones	*Leiurus quinquestriatus hebraeus*	Deathstalker
	Centruroides exilicauda	California bark scorpion
Lepidosauria (scaled lizards)	*Heloderma suspectum*	Gila monster

Table 1. Animal venoms that have been shown to be detoxified by mast cells or mast cell proteases ([175, 177, 181, 182] and unpublished data)

Figure legends

Fig. 1. Role of MC in immune reactions and physiological tissue remodeling. Located at potential entry sites of harmful agents, they are able to recruit and activate effector immune cells, but also to exert direct, e.g. antimicrobial effects. They are crucial for uterine and spiral artery remodeling in pregnancy. Pro-angiogenic functions of activated MC are perceived as a double edged sword as MC have also been shown to enhance tumor vascularization.

Fig 2. MC orchestrate tissue resident immune cell functions and recruitment of additional innate and adaptive effector cells. Due to the immediate response to danger or infection, MC initiate vascular responses and infiltration of neutrophils and effector T cells, partially in conjunction with macrophages. MC promote DC migration and maturation via soluble mediators and physical interactions. Hence, MC impact on LN-borne induction of adaptive immunity i.e. priming of effector T cells via modulation of DC functionality. Importantly, the dynamic interaction between MC and DC culminates in protein exchange towards MC thereby impacting on MC functions. The cross-dressing of MC with MHCII complexes by DC equips them with antigen-presenting capacity resulting in MC-driven activation of homing effector T cells.

Fig 3. MC contributions to immune defense against infection. **A**, Interactions of MC with *Staphylococcus aureus*. Colorized electron photographs showing *S. aureus* (yellow) attached to MC (left panel) and entrapped in the anti-microbial extracellular traps released by MC (right panel). **B**, Leishmania attached to MC in skin lesions. **C**, The multitude of MC effects contributing to host defense against bacterial, viral and parasite pathogens. MC contribute to the clearance of bacterial infections by direct antimicrobial response via cathelicidin, phagocytosis and trap formation and of viral infections again by cathelicidin. In addition, invading parasites are directly attacked by MC via release of chymase, tryptase and reactive oxygen species (ROS). On the other hand, MC support the host defense against bacterial,

parasite or viral infections by the recruitment of further innate and adaptive immune cells, i.e. neutrophils; Th2 cells and eosinophils; and NK cells, NKT cells, and cytotoxic T cells, respectively. During parasite infection, MC enhance adaptive response by modulating DC migration and activation.

Fig 4. The role of mast cells for reproductive processes. Maturation and activation of uterine mast cells (uMC) that consist of mucosal-type MC (MMC) and connective tissue-type MC (CTMC) can be influenced hormonally by estradiol and progesterone. MC activation results in the release of numerous preformed or newly synthesized mediators including histamine, tryptases, chymases, and many others. They are directly or indirectly involved in processes like implantation, angiogenesis, defense against pathogens and uterine remodeling that are in turn important for pregnancy success. The MC protease α-chymase (MCP-5) positively influences spiral artery (SA) remodeling by activating vascular smooth muscle cell (VSMC) apoptosis and extravillious trophoblast (EVT) migration. Sufficient SA remodeling is important for placental and fetal development, whereas impaired SA remodeling is associated with preeclampsia, intrauterine growth restriction (IUGR), preterm birth and miscarriage. Also the interaction and communication of uMC with uterine natural killer cells (uNKs), regulatory T cells (Tregs) and trophoblasts are substantial for pregnancy maintaining.

Figure 1

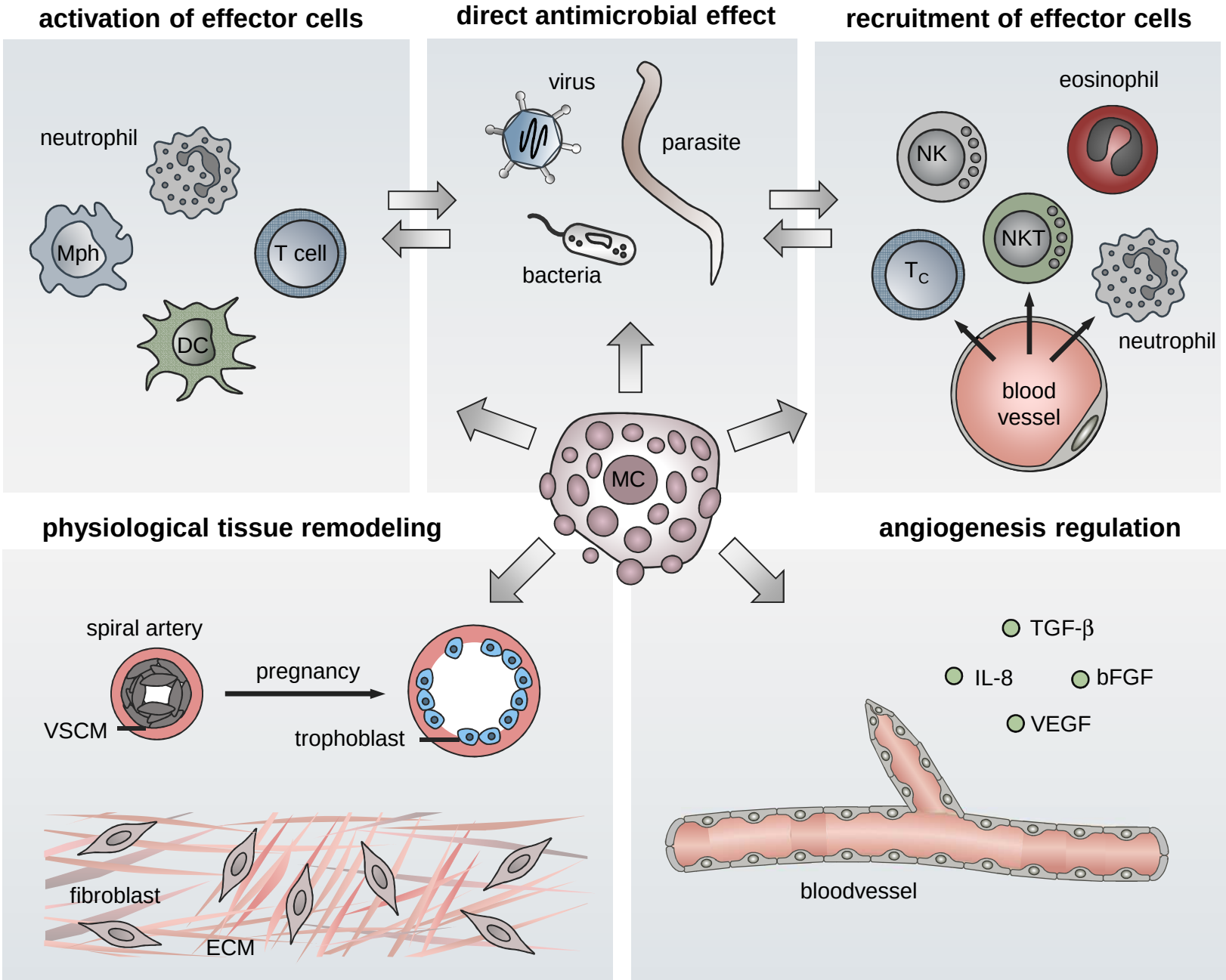

activation of effector cells
direct antimicrobial effect
recruitment of effector cells
neutrophil
Mph
T cell
DC
virus
parasite
bacteria
eosinophil
NK
NKT
T_C
neutrophil
blood vessel
MC
physiological tissue remodeling
angiogenesis regulation
spiral artery
pregnancy
VSCM
trophoblast
fibroblast
ECM
TGF-β
IL-8
bFGF
VEGF
bloodvessel

Figure 2

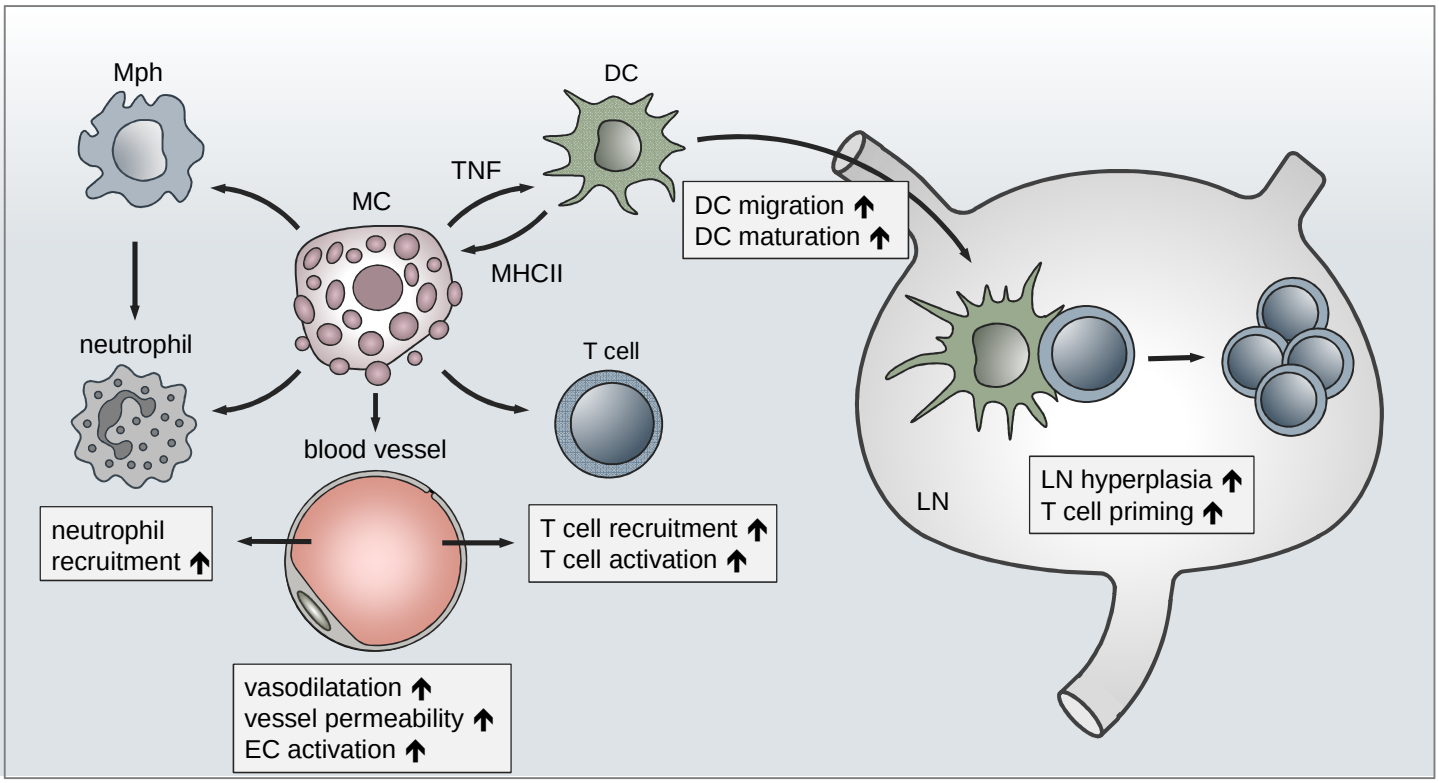
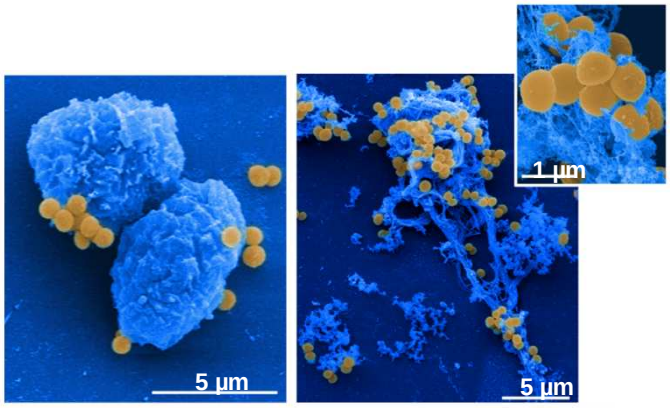
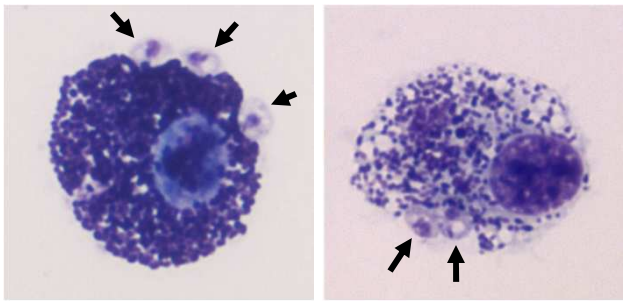

Mph
DC
TNF
MC
DC migration
DC maturation
MHCII
neutrophil
T cell
blood vessel
LN
LN hyperplasia
T cell priming
neutrophil
recruitment
T cell recruitment
T cell activation
vasodilatation
vessel permeability
EC activation

Figure 3

A


5 µm
5 µm
1 µm

B



C

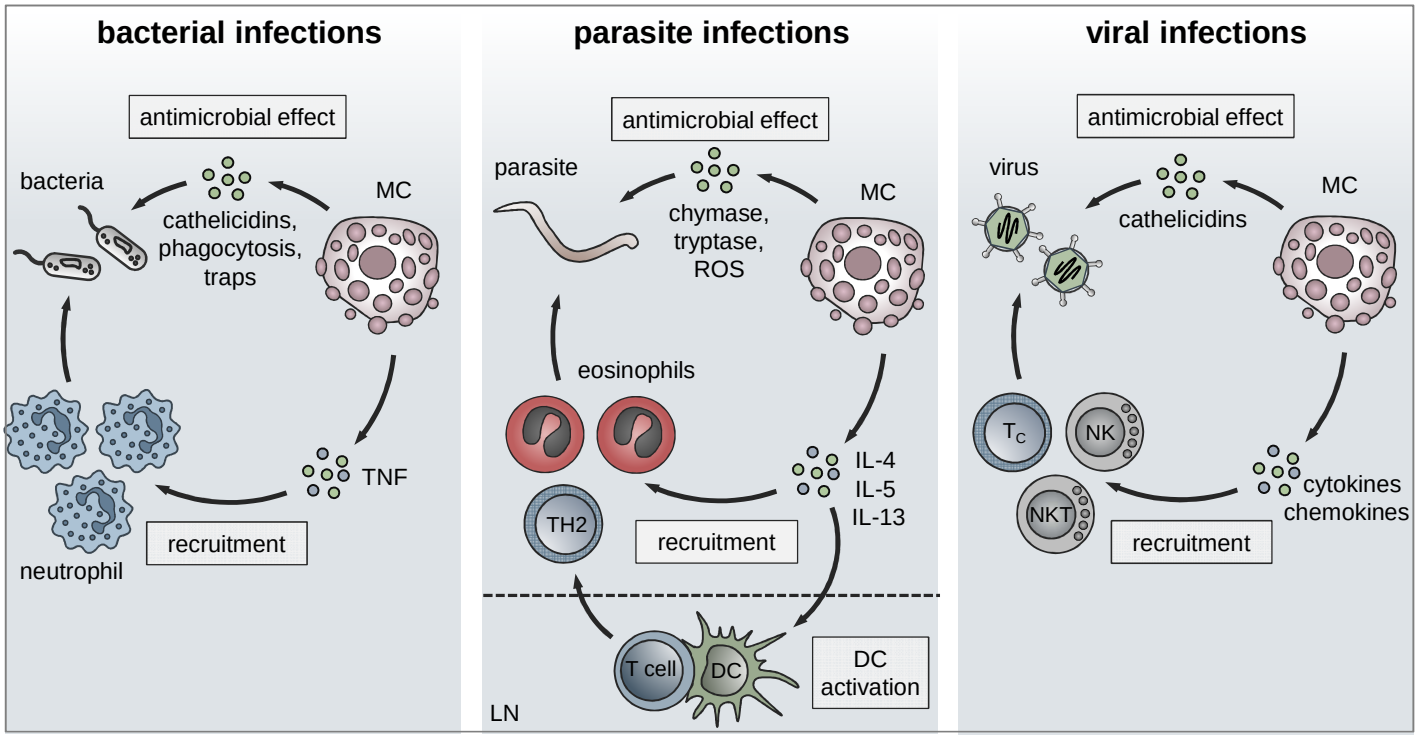
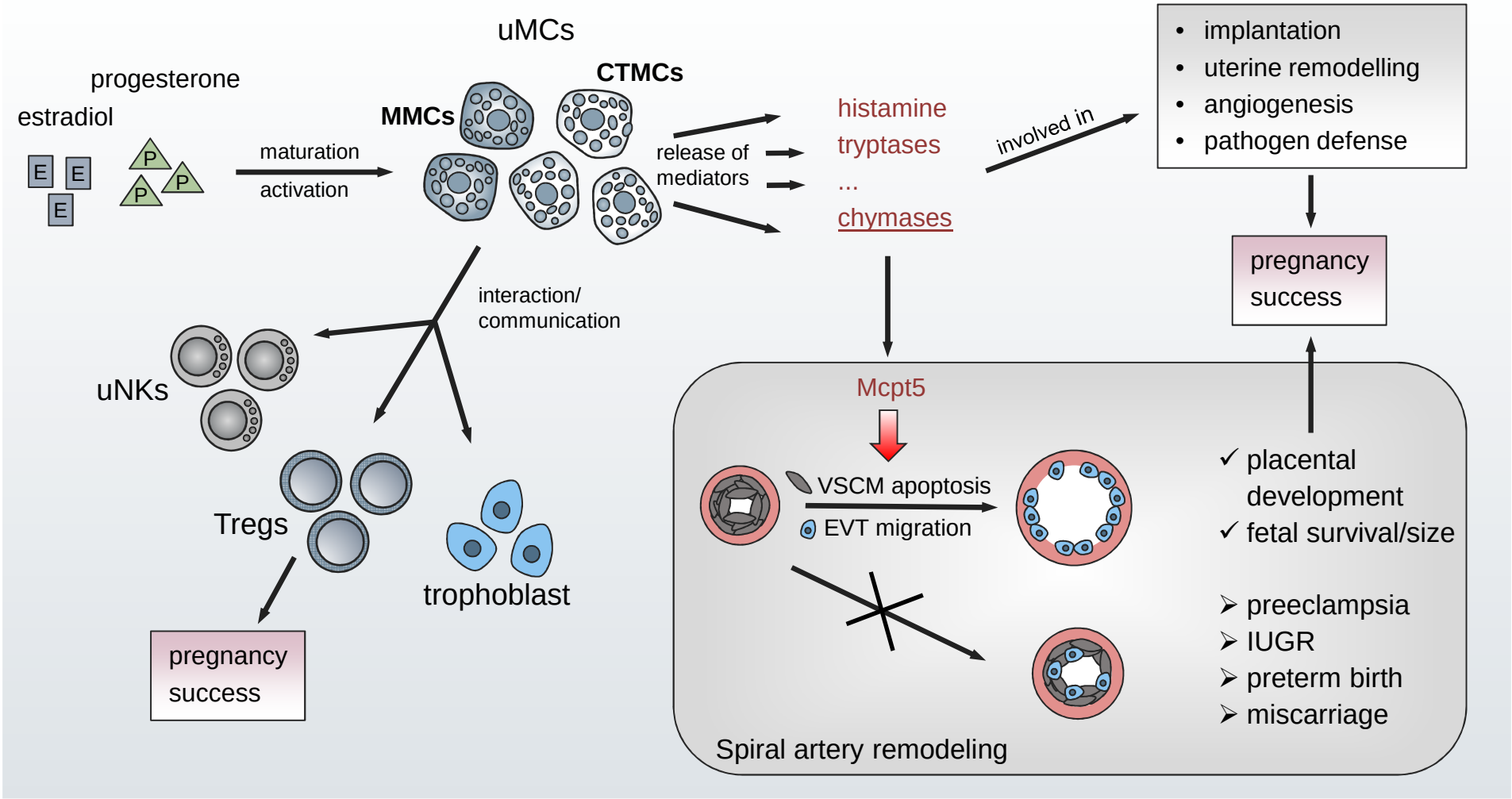

bacterial infections
antimicrobial effect
bacteria
cathelicidins,
phagocytosis,
traps
MC
TNF
recruitment
neutrophil
parasite infections
antimicrobial effect
parasite
chymase,
tryptase,
ROS
MC
eosinophils
IL-4
IL-5
IL-13
TH2
recruitment
T cell
DC
DC
activation
LN
viral infections
antimicrobial effect
virus
cathelicidins
MC
T_C
NK
NKT
cytokines
chemokines
recruitment

Figure 4


uMCs
progesterone
estradiol
E
E
E
P
P
P
maturation
activation
MMCs
CTMCs
release of
mediators
histamine
tryptases
...
chymases
involved in
• implantation
• uterine remodelling
• angiogenesis
• pathogen defense
pregnancy
success
interaction/
communication
uNKs
Tregs
trophoblast
pregnancy
success
Mcpt5
VSCM apoptosis
EVT migration
✓ placental
development
✓ fetal survival/size
➢ preeclampsia
➢ IUGR
➢ preterm birth
➢ miscarriage
Spiral artery remodeling