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Mast cells within cellular networks

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Abstract

Mast cells are highly versatile in terms of their mode of activation by a host of stimuli and their ability to flexibly release a plethora of biologically highly active mediators. Within the immune system, mast cells can best be designated as an active nexus interlinking innate and adaptive immunity. Herein, we try to draw an arc from the initiation of acute inflammatory reactions to microbial pathogens to the development of adaptive immunity and allergies. This multifaceted nature of mast cells is made possible by the interaction with multiple cell types of immunological and non-immunological origin. Examples for the former include neutrophils, eosinophils, T cells and professional antigen presenting cells. These interactions allow mast cells to orchestrate inflammatory innate reactions and complex adaptive immunity, including the pathogenesis of allergies.

Important partners of non-immunological origin include cells of the sensory neuronal system. The intimate association between mast cells and sensory nerve fibers allows bidirectional communication leading to neurogenic inflammation. Evidence is accumulating that this mast cell / nerve crosstalk is of pathophysiological relevance in allergic diseases like asthma.

44 **Key words**

45

46 Mast cells, neutrophils, eosinophils, T cells, asthma, mCMV infection, vaccination, substance

47 P, sensory nerves, neurogenic inflammation

48

Abbreviations

- APC: Antigen presenting cell
- BALF: Bronchoalveolar lavage fluid
- BMMC: Bone marrow-derived mast cells
- c48/80: Compound 48/80
- CADM1: Cell adhesion molecule 1
- CGRP: Calcitonin gene-related peptide
- CHSR: Contact hypersensitivity reaction
- CTL: Cytotoxic T lymphocyte
- CUC: Chronic ulcerative colitis
- EAE: Experimental autoimmune encephalomyelitis
- HDM: House dust mite
- IBD: Inflammatory bowel disease
- mCMV: Murine cytomegalovirus
- MRGPRX2: Mas related G protein coupled receptor
- NGF: Nerve growth factor
- PGD2: Prostaglandin D2
- ROS: Reactive oxygen species
- SCG: Superior cervical ganglia
- SP: Substance P
- TNF: Tumor necrosis factor alpha
- Treg: Regulatory T
- Trk: Tropomyosin-related kinase
- TRPV1: Transient receptor potential vanilloid type 1
- VIP: Vasoactive intestinal peptide

Interactions of mast cells with neutrophils and eosinophils

Neutrophils comprise 50-70% of human leukocytes in peripheral blood and $1-2 \times 10^{11}$ cells per day are continuously generated in the bone marrow. A key step of an acute inflammatory response is the rapid recruitment of neutrophils from the blood to the infected tissues where they can set up a first line of defense as effector cells against microbial invaders^{1,2}. There is ample evidence that both the recruitment of neutrophils as well as some of their effector functions can be promoted by activated mast cells. Initially, it was shown in several murine models for acute inflammation that mast cells are able to initiate the recruitment of neutrophils³⁻⁹. A cornerstone in this context were the observations that mast cells trigger the life-saving influx of neutrophils in murine models for acute bacterial infections¹⁰⁻¹². With regard to the underlying mechanisms, mast cell-derived TNF, which can be stored within secretory granules and released on demand¹³, plays a prominent role for attracting neutrophils. Additionally, mast cell-derived TNF was reported to be critical for neutrophil recruitment in late-phase cutaneous anaphylaxis¹⁴, contact hypersensitivity reactions (CHSR)^{15,16}, Th17 cell-dependent lung inflammation¹⁷, formation of cutaneous granulomas¹⁸, IL-33-induced peritonitis¹⁹ and experimental autoimmune encephalomyelitis (EAE)²⁰. Of note, this latter finding was not reproduced by using a Kit-independent strain of mast cell-deficient mice that avoids the pleiotropic effects of Kit mutations across a variety of cells beyond the mast cell itself²¹.

In general, there is ample evidence that the nature of the mutation resulting in mast cell-deficiency, the strain background of mice and the protocol to induce an experimental phenotype all can contribute to the generation of contradictory results when comparing different mast cell-deficient mouse strains. For a detailed review of this subject, the reader is referred to²²⁻²⁵.

Besides TNF, mast cell-derived leukotrienes (LT), human tryptase, mouse tryptase mast cell protease 6 (mMCP-6) and macrophage inflammatory protein 2 (MIP-2) are critical for the

protective influx of neutrophils²⁶⁻³⁰. In a murine CHSR model it was reported that recruitment of neutrophils is driven by mast cell-derived TNF acting on endothelial cells expressing TNFRI³¹. Taken together, there is ample evidence that the attraction of neutrophils as an important step in a variety of acute inflammatory reactions can be mediated by mast cell-mediators including TNF.

Part of our own work focused on the impact of mast cell mediators on the activation of murine neutrophil effector functions³². Cocultures of IgE/antigen-activated murine bone marrow-derived mast cells (BMMC) and neutrophils freshly isolated from bone marrow revealed that activated mast cells strongly modulate the expression of neutrophil activation markers, i.e. decrease of CD62L and increase of CD11b, on the neutrophil surface. Furthermore, activated BMMC initiate the generation of reactive oxygen species (ROS) in neutrophils, enhance their phagocytosis and promote neutrophil survival in vitro. Mechanistically, all these phenomena partly depend on the mast cell-derived cytokines TNF and, to an even greater extent, on GM-CSF, and do not require cell-cell contact. In an LPS-driven murine model for acute lung inflammation we demonstrated that neutrophil phagocytosis is impaired in mast cell-deficient *Kit^{W-sh}/Kit^{W-sh}* mice. Consequently, phagocytosis can be restored upon engraftment with wild type-derived but not with GM-CSF-deficient mast cells, corroborating our in vitro findings³². Thus, mast cells can contribute to the local activation of critical neutrophil effector functions as well as to their prolonged survival which may be facilitated by close cell-to-cell contact³³.

It is tempting to speculate that neutrophils may also have an impact on the activation of mast cells in a bi-directional communication between these cell types, yet this deserves further investigation.

In this context, more information has been gathered on the interactions between mast cells and eosinophils. Infiltrating eosinophils have been detected in close proximity to mast cells in allergic inflamed tissue and in several other diseases (reviewed by^{34,35}). This led to the

hypothesis that mast cells and eosinophils form the "allergic effector unit"³⁶⁻³⁸. Within this "allergic effector unit", both cell types are suggested to have soluble and physical crosstalk that enhances cellular functions amplifying the late phase of allergic inflammation. Mast cell-derived soluble factors able to modulate eosinophil function include IL-5, GM-CSF and IL-3, among others. Interestingly, cocultures of eosinophils and mast cells prolonged the otherwise poor survival of the former in the presence of dexamethasone. This effect may play a role in corticosteroid-resistant asthmatic patients³⁶. On the other hand, eosinophils are able to release a variety of factors able to promote the activation of mast cells, including platelet-activating factor, stem cell factor, nerve growth factor, eosinophil major basic protein, eosinophil cationic protein and eosinophil peroxidase (see³⁴ for review). Furthermore, eosinophils enhanced mast cell mediator release through physical contact involving interaction between CD48 expressed on mast cells and 2B4 on eosinophils³⁸. Taken together, these paracrine and physical interactions between both cell types may be relevant in allergic diseases and also in several inflammatory and neoplastic disorders³⁵.

However, besides interactions with cells from the innate part of the immune system, mast cells are also able to communicate with cells from the adaptive immune system, as outlined in the following two sections.

Crosstalk between effector T cells and mast cells

Close proximity between mast cells and T cells in inflamed tissues led to the suggestion of a bidirectional relationship between these cell populations (reviewed by^{39,40}). In general, interactions of T cells and mast cells can be cell-to-cell-, cytokine- and exosome-mediated⁴¹. In the absence of soluble mediators, direct contact between human and murine mast cells and activated T cells was reported to induce mediator release and activation of mast cells, causing the release of stored mediators and production of cytokines including TNF, IL-4, IL-6 and IL-8⁴²⁻⁴⁶. Interactions between intercellular adhesion molecule-1 (ICAM-1) and lymphotoxin- β

receptor (LT β R) with their respective ligands was reported to be responsible for T cell-induced mast cell activation⁴². Activation of mast cells could also be accomplished upon incubation with cell membranes derived from activated T cells^{43,47}. Additionally, surface molecules on membrane vesicles secreted by T cells were also shown to activate mast cells⁴⁸. The latter phenomenon may allow a distant activation of mast cells.

For the stimulation of effector T cells by mast cells, the ability of the latter to present antigens and to express costimulatory molecules is of central importance. A combination of LPS and IFN- γ was shown to induce both the expression of MHC class II molecules and of costimulatory CD80/CD86 molecules on mast cells^{49,50}. Inducible expression of MHC II, processing and presentation of antigenic peptides on mast cells leads to the expansion of previously activated CD4⁺ T effector cells and also of CD4⁺CD25⁺ regulatory T cells (Treg). Moreover, activated mast cells can promote T cell activation by soluble TNF⁵¹, by triggering the costimulatory molecule OX40⁵², and via the release of exosomes carrying MHC class II molecules and CD86⁵³. Synapse-like contacts between dendritic cells and mast cells initiated by inflammatory conditions lead to the transfer of MHC II complexes, enabling the latter to prime allogeneic T cells⁵⁴. In the human system, mast cells primed with IFN- γ are able to induce superantigen-specific T-cell responses and antigen-specific Th1 recall responses⁵⁵. However, mast cells should not be regarded as professional antigen presenting cells (APC) able to activate naive T cells but they can function as nonprofessional or atypical APC⁵⁶.

Evidence has accumulated suggesting a protective role for mast cells in antiviral immune responses⁵⁷⁻⁵⁹. In this context, we have shown that mast cells degranulate upon infection of mice with murine cytomegalovirus (mCMV) involving TLR3/TRIF-dependent and independent mechanisms⁶⁰. Upon infection, mast cells are also critical for the recruitment of mCMV-specific CD8 T cells to the lungs⁶¹. The lungs are a predilection site for mCMV infections and, accordingly, interstitial pneumonia is the most dreaded manifestation of CMV disease in immunocompromised patients. In mast cell-deficient *Kit*^{W-sh}

mice, CD8 T cells were recruited less efficiently to the lungs, correlating with strongly enhanced viral replication and delayed clearance of the virus. Both phenomena were restored upon reconstitution of mast cell-deficient mice with mast cells generated in vitro^{61,62}.

Most likely, a wave of mast cell-derived CC chemokine ligand 5 (CCL5) in the serum of infected mice contributes to the recruitment of mCMV-specific CD8⁺ T cells, in agreement with published findings⁶³. However, in the mCMV model, mast cell-deficiency does not affect functionality and expansion of virus-specific CD8⁺ T cells⁶¹.

Impact of mast cells on professional APC in models for vaccination and allergic airway disease

Initially, it was shown that mast cells are able to trigger the migration of professional antigen presenting cells in several murine models⁶⁴⁻⁶⁸. Concomitantly, mast cells enhance the expression of costimulatory molecules on APC (CD80, CD86, CD40), thereby also promoting their maturation (recently reviewed by)⁶⁹. Thus, by influencing both migration and maturation of APC, mast cells are well suited to modulate APC-driven immune responses, acting at the interface of innate and adaptive immunity.

This property of mast cells as a nexus linking innate and adaptive responses makes them an attractive target in vaccination models employing adjuvant in combination with antigen. In this context, we demonstrated that mast cells are critical for the development of a variety of phenomena following topical application of the TLR7 ligand imiquimod in combination with a CTL antigenic peptide, i.e. a murine model for transcutaneous immunization⁷⁰. Mast cells expressing TLR7 and mast cell-derived cytokines IL-1 β and TNF are critical to mount a rapid and full-blown inflammatory response upon application of adjuvant, mainly characterized by the influx of neutrophils^{70,71}. Additionally, hypertrophy of draining lymph nodes is driven by mast cell-derived TNF, in agreement with published data^{16,72,73}. Emigration of Langerhans

cells as professional APC from the epidermis to the draining lymph nodes is partly dependent on mast cell-derived IL-1 following topical application of imiquimod.

Finally, the expansion and function of peptide-specific cytotoxic CD8⁺ T cells is severely impaired in this model in the absence of mast cells⁷⁰. In line with this, the mast cell activator compound 48/80 (c48/80) was also shown to exert a mucosal⁷⁴ and intradermal⁷⁵ adjuvant function and to enhance protective humoral immunity. Of note, adjuvant effects of c48/80 applied either intradermally or via the mucosal route were recently shown to be independent of mast cells using Kit-independent mast cell-deficient mouse strains⁷⁶.

Murine inflammatory airway disease based on sensitization to antigens and subsequent provocation is often used as a model for the pathogenesis of human asthma, including inflammation of the airways, overproduction of mucus and hyperreactivity to bronchoconstrictors⁷⁷. Mast cells have been linked to the induction of allergic airway disease in mice, although this was shown to depend on the experimental model⁷⁸⁻⁸⁰. In this context, we were able to demonstrate that, in previously sensitized mice, mast cells can promote the migration of antigen-laden APC from the lung to the draining lymph nodes upon intranasal challenge with the antigen⁶⁷. Accordingly, in the absence of mast cells, airway inflammation, production of mucus and airway hyperreactivity were all decreased. Interestingly, the phenotype seen in mast cell-deficient mice could be restored following transfer of antigen-laden APC to sensitized mice prior to airway challenge. Taken together, this study suggests that the interaction between mast cells and APC can be critical for the development of airway disease.

However, we were able to show that the contribution of mast cells to the development of murine allergic airway disease strongly depends on the genetic background⁸¹. Regarding the latter, identification of a traceable polymorphism closely linked to the *Kit*^{W-sh} allele allowed us to generate congenic mast cell-deficient mice on BALB/c background, termed C.B6-*Kit*^{W-sh}⁸¹. Unexpectedly, and in sharp contrast to their C57BL/6-*Kit*^{W-sh} mast cell-deficient

counterparts, C.B6-Kit^{W-sh} mice develop all signs of allergic airway disease despite the absence of mast cells. This phenomenon may, at last partly, reflect the complexity seen in human disease. In the next section, we will shed some light on mast cell / nerve interactions and how it contributes to physiologic and pathophysiologic responses with a focus on allergic inflammation.

Mast cell interactions with the sensory neuronal system in chronic inflammatory diseases

Microlocalization is a fundamental organizing principle of inflammatory responses, since many neuronal and inflammatory mediators are rapidly inactivated. The functional communication between mast cells and nerves depends on membrane-membrane contacts mediated by cell adhesion molecule 1 (CADM1)^{82,83}. The intimate association was shown by the presence of dense core vesicles at the points of contact⁸⁴. Close anatomical proximity and functional communication of mast cells with sensory nerves has been suggested in different organs such as gut⁸⁵, skin⁸⁶ and respiratory tract⁸⁷⁻⁹⁰. In chronic skin diseases, including psoriasis and atopic dermatitis, the contact between mast cells and sensory nerves are increased in number⁸⁶. Many inflammatory diseases in skin and intestine are accompanied by mast cell hyperplasia in the inflamed mucosal epithelium and in the adjacent smooth muscle^{91,92}. Therefore the mast cell / nerve communication in smooth muscle may be enhanced. Human skin mast cells have shown immunoreactivity to the neuropeptide substance P (SP). Using electron microscopy, Toyoda et al. demonstrated that SP was localized within the mast cell secretory granules. Interestingly, mast cells positive to SP and the amount of SP within mast cells were increased in lesional skin from atopic patients⁹³. This is suggesting that SP could be synthesized as a newly generated mediator when inflammation prevails. *In vivo* experiments using human skin have shown that intradermal injection of SP provokes weal, flare and itching. These responses are mainly mediated by mast cell degranulation, followed

by increased vascular permeability and marked infiltration of eosinophils and neutrophils and can be blocked upon depletion of peripheral nerve neuropeptide content⁹⁴⁻⁹⁶. Moreover, Stoyanova and Gulubova described a population of mast cells in the human intestine being immunopositive for both tryptase and SP. These cells were found in higher frequencies in biopsies from patients with chronic ulcerative colitis (CUC) compared with controls⁹⁷. In biopsies from the gut of patients with inflammatory bowel disease (IBD), but not in biopsies from non-inflamed tissue, SP treatment induces secretion of histamine⁹⁸. In our group we analyzed the distribution and localization of mast cells and sensory nerve fibers in cross-sectioned small airways using human precision-cut lung slices. We showed a close anatomical localization of mast cells with sensory nerves, especially with mast cells containing the neuropeptide substance P (Fig. 1). These observations indicate that an inflammatory environment increases the frequencies of SP and tryptase double-positive mast cells. Nevertheless, whether human mast cells produce SP has to be confirmed on the mRNA level. Indeed, several lines of evidence point out that functional communication between mast cells and sensory nerves is involved in the pathogenesis of chronic inflammatory diseases.

Vagal sensory neurons represent a dominant afferent innervation to the airways and lungs^{99,100}. C-fibers are a non-myelinated subset of afferent nerves that have been shown to be involved in the pathophysiology of asthma including airway hyperreactivity¹⁰⁰⁻¹⁰². C-fibers are characterized by co-expression of the transient receptor potential vanilloid type 1 receptor (TRPV1) ion channel and sensory neuropeptides like SP¹⁰³. Interestingly, they serve not only as afferent information wires, but they have a strong local effector function by releasing neuropeptides on the site of stimulation (axon reflex). Upon stimulation with inhaled irritants or endogenous signals associated with tissue inflammation, C-fibers release neuropeptides via the axon-reflex mechanism leading to neurogenic inflammation. In this setting, neuropeptides affect neighboring effector cells leading to bronchoconstriction, protein extravasation, mucus

secretion and recruitment of leukocytes¹⁰⁴. The neuropeptide SP is known as one of the most important mediators of neurogenic inflammation. Increased levels of SP have been detected in BALF¹⁰⁵, sputum^{106,107} and plasma¹⁰⁸ from asthmatic patients and increased levels of SP have also been found upon allergen challenge. SP is an effective constrictor of airways and this has been demonstrated in *in vitro* and *in vivo* experiments using different animals and human airways¹⁰⁹⁻¹¹². SP has a high affinity for the NK1 receptor and it also binds, but with lower affinity, to the other tachykinin receptors NK2R and NK3R¹¹³. Besides the direct effect of SP on NK1R on airway smooth muscle cells, several studies have suggested that the SP/NK1R route can contribute to airway constriction indirectly by the activation of mast cells^{109,114}.

The theory that, under allergic conditions, NK1R expression has a functional significance for asthma symptoms and affects the sensitivity of mast cells to SP was challenged by the failure of several NK1R antagonists in clinical studies^{115,116}. This was explained by limited pharmacological properties of the antagonists, the complexity of the neuropeptide system including redundant neuropeptides and receptors as well as with alternative receptor pathways. Regarding the latter, it was demonstrated that SP activates murine mast cells and human tryptase- and chymase positive mast cells (MC_{TC}) through mas related G protein coupled receptor X2 (MRGPRX2)¹¹⁷⁻¹¹⁹. Most MC_{TC} were found in human connective tissues, such as the skin^{120,121}. Brightling et al showed in bronchial biopsies that infiltration of the airway smooth muscle cell (SMC) layer with MC is associated with airway disorder. In a subgroup of subjects with asthma these MCs were tryptase and chymase positive¹²². Increased frequencies of MC_{TC} and MRGPRX2-positive mast cells in the lungs of asthma patients indicate that an MRGPRX2-expressing MC_{TC} subpopulation may influence asthma pathology^{123,124}. However, the exclusive expression of MRGPRX2 on MC_{TC} in the lung and its function in airway hyperresponsiveness has to be proven. Other studies demonstrated that intraepithelial mast cells in patients with T(H)2-high asthma have a different phenotype

containing high levels of tryptase and carboxypeptidase 3 (CPA3) but low chymase levels, and predict responsiveness to inhaled corticosteroids ¹²⁵. These studies demonstrate the plasticity of mast cell phenotypes that may change under pathological conditions according to the specific microenvironment ¹²⁶. In our own work (unpublished) we showed that the challenge of sensory nerve endings leads to an axon reflex response which activates mast cells via substance P. Ultimately, this causes enhanced airway constriction in passively sensitized human lung tissue. Enhanced airway constriction was inhibited by the NK1R-specific antagonist fosaprepitant (IVEMEND) ¹²⁷. The expression of NK1R on mast cells seems to be regulated by IgE binding to Fc_εRI as shown in several studies with *in vitro* cultures and confirmed by our group ^{128,129,130}. Using fresh human lung tissue, we show that anti-IgE antibody treatment (Omalizumab) during passive sensitization of lung tissue leads to a significant decrease of neuronally-induced bronchoconstriction. These data emphasize that IgE binding to mast cells positively affects the expression of NK1R on mast cells and thus their sensibility to SP. However, SP was shown to induce mast cell degranulation ¹³⁰⁻¹³² and histamine release from a variety of mast cells types, including human mast cells isolated from BALF ^{133,134}, skin ¹³⁵ and inflamed gut mucosa ⁹⁸. However, the presence of NK1R and MRGPRX2 on mast cells suggest autocrine as well as paracrine mechanisms. For instance, it has been reported that SP induces release of cytokines in human mast cells ¹³⁰.

It has to be mentioned that neuropeptides also have anti-inflammatory and protective functions. It should be recalled that mast cell degranulation and inflammation can also be negatively regulated, e.g. by the calcitonin gene-related peptide CGRP or vasoactive intestinal peptide/pituitary adenylate cyclase activating peptide (PACAP) family, which often co-localize with SP ^{136,137}. In a murine model of allergic asthma, CGRP inhibits DC maturation and allergen-specific T cell responses, which affects the outcome of allergic airway inflammation *in vivo*. In the gut, the neuropeptide CGRP exhibits protective effects on gastric mucosa injury after cerebral ischemia reperfusion in rats ¹³⁸. A regulatory interaction between

SP and CGRP was shown when injection of CGRP with substance P into human skin converts the long-lasting vasodilatation induced by CGRP into a transient response. Vasodilation was regulated by the action of proteases from mast cells stimulated by substance P¹³⁹.

Mast cells synthesize and release a variety of molecules which, in turn, influence neuronal activity. Mast cell mediators including tumor necrosis factor (TNF), tryptase, nerve growth factor (NGF), prostaglandin D2 (PGD2) and adenosine can enhance the sensitivity of TRPV1 or lower its threshold for activation¹⁰². Therefore, allergic reactions in the airways may increase the excitability of sensory fibers and promote a feedback between mast cells and terminal nerves¹⁰¹. Moreover, this effect can increase parasympathetic tone in the airways by increasing central and peripheral reflex arcs⁸⁹. Indeed, increasing evidence indicates that TRP receptors like the TRPV1 channel are relevant in the development of several aspects of airway hypersensitivity¹⁰². Thus, mast cells and nerves mutually influence each other in different pathologies including psoriasis, IBD, and asthma.

Nerve growth factors like NGF seem to play a special role in the mast cell / nerve interaction especially in setting the sensitivity of the interaction and inducing hyperreactivity in pathophysiological conditions. Neurotrophins have been shown to promote differentiation and survival of mast cells, which in turn represent a major source of neurotrophins. In diseases like mastocytosis the neurotrophin system is highly upregulated. In combination with increased neurotrophin levels, the elevated expression of several isoforms of tropomyosin-related kinase (Trk) receptors on mast cells contributes to the pathophysiology of mastocytosis in autocrine and paracrine loops¹⁴⁰. In skin, mast cells act as a "power house" by releasing algogenic and pruritogenic mediators, which initiate a reciprocal communication with specific nociceptors on sensory nerve fibers. Consequently, nerve fibers release inflammatory and vasoactive neuropeptides, which in turn activate mast cells in a feedback mechanism, thus promoting a vicious cycle of mast cell and nociceptor activation leading to

neurogenic inflammation and pain/pruritus¹⁴¹. In this context, we were able to show in a rat asthma model that the mast cell-dependent early phase response can be blocked by anti-NGF treatment¹⁴².

The interaction between mast cells and sensory nerves is occurring preferentially in organs with direct contact to the environment like skin, lung and gut. For instance, in the lung these interactions are of pathophysiological relevance in allergic diseases like asthma. However, the underlying mechanisms are not completely resolved.

Conclusions

With the advent of mast cell-deficient mouse strains and their widespread use, functions of mast cells have been considered to be much broader than their original role in IgE-driven immediate-type reactions. However, caveats have been raised about mast cell-deficient Kit-mutant mice and their reconstitution with mast cells generated *in vitro*.

Ideally, Kit-dependent and -independent strains deliver consistent results which are corroborated by findings derived from human mast cells *ex vivo* or *in vitro*. In the future, mouse strains that allow the efficient development of human mast cells from hematopoietic stem cells may be extremely useful tools for investigation of mast cell biology and as preclinical models¹⁴³.

In different studies it has become apparent that mast cells can be critical for the rapid recruitment and activation of neutrophils, i.e. for the induction of an acute inflammatory response (Fig. 2). Furthermore, there is ample evidence that mast cells can promote the induction of adaptive immune responses in different ways. First, mast cells can support migration and maturation of professional APC to the draining lymph nodes. Second, mast cells can directly interact with T cells. In this context, mast cells can act as nonprofessional APC by presenting antigen to preactivated T cells, either after uptake and processing of antigen or after receiving MHC II from professional APC upon cell-to-cell interaction.

Intimate interactions between peripheral sensory neurons and mast cells form a microenvironment for communication resembling synaptic junctions. Emerging evidence indicates that mast cell mediators can act on neurites and *vice versa*. The axon reflex mechanism allows the activation of mast cells by neuropeptides, leading to neurogenic inflammation including contraction of smooth muscle cells, mucus secretion and recruitment of leukocytes in chronic inflammatory diseases. Using novel and more specific models it will

393 be fascinating to further unravel the manifold functions and interactions of mast cells with
394 immune and non-immune cells.
395

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Figure legends

FIG 1. Distribution and localization of mast cells and sensory nerve fibers in cross-sectioned small airways of human precision-cut lung slices. Human lung material was cut into 300µm thick viable precision-cut lung slices including cross-sectioned small airway. Nerves were immunostained with the pan neuronal marker PGP9.5 (red), mast cells were visualized with anti-human tryptase antibody (yellow) and SP was localized with an anti-SP antibody (blue). Lung tissue autofluorescence was used to illustrate lung structure. A: A representative image illustrating the airway lumen (Al) epithelium (Ep) and submucosa (S) in a cross-sectioned small airway shows distribution of peripheral nerves (red), mast cells (yellow), and the neuropeptide substance P (SP, blue). SP-positive sensory nerves (pink) were observed in proximity to SP-positive mast cells (white). Three-dimensional images. Scale bar 30µm. B: Zoom of the indicated rectangular area in A, showing subepithelial mast cell / nerve interactions including sensory nerves and SP and tryptase double-positive mast cells. Scale bar 10 µm. Al: airway lumen. Ep: airway epithelium; S: submucosa.

FIG 2. The networking mast cell

Afferent sensory nerves (C-fibres) can be activated by exogenous or endogenous stimuli. This sets up an axon reflex mechanism leading to neurogenic inflammation. Secreted neuropeptides such as SP released via the axon reflex mechanism activate mast cells by binding to NK receptors or MRGPRX2 to release mediators (histamine, LTC₄) promoting the contraction of smooth muscle cells. Vice versa, activation of afferent neurons by mast cell-derived mediators (tryptase, histamine, cysteinyl leukotrienes and neurotrophins) causes neuronal hyperreactivity and thus a vicious circle of neuroinflammation.

Mast cells activated by IgE or IgE-independent signals are able to promote the migration of antigen-laden professional APC to the draining lymph nodes where they initiate the expansion

of antigen-specific T cells. Additional signals including mast cell-derived TNF are required to facilitate this initiating event in the development of the adaptive immune response. Thereby, it is very likely that mast cells have an impact on the magnitude of the immune response. Mast cell mediators such as CCL5/RANTES have a role in the recruitment of CD8⁺ T cells to sites of viral infection. The activation of T effector cells and mast cells can also be triggered by intercellular crosstalk. In this context, mast cells can function as nonprofessional APC. Additionally, mast cells are able to activate endothelial cells leading to the attraction of neutrophils as a critical event in many acute inflammatory reactions. Moreover, mast cells also boost the activation of neutrophils.

Fig. 1

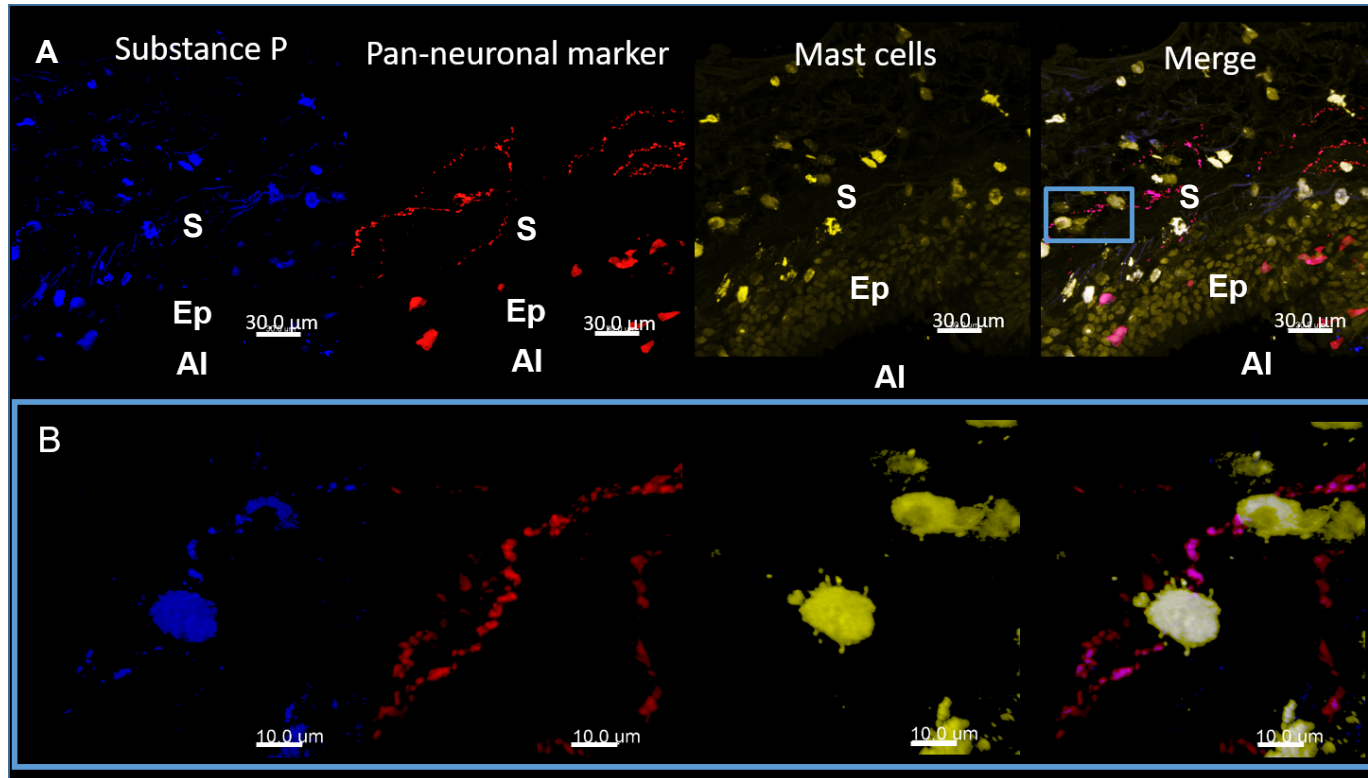


Fig. 2

