

Imaging of mast cells

Shweta Tikoo^{1,2}  | Natasja Barki³ | Rohit Jain^{1,2}  | Nursafwana S. Zulkhernain¹ | Sabine Buhner³ | Michael Schemann³  | Wolfgang Weninger^{1,2,4} 

¹The Centenary Institute, Newtown, NSW, Australia

²Discipline of Dermatology, Sydney Medical School, Sydney, NSW, Australia

³LS Human Biology, Technical University München, München, Germany

⁴Department of Dermatology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

Correspondence

Michael Schemann, LS Human Biology, Technical University München, München, Germany.

Email: schemann@wzw.tum.de and

Wolfgang Weninger, The Centenary Institute, Newtown, NSW, Australia.

Email: w.weninger@centenary.org.au

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Summary

Mast cells are a part of the innate immune system implicated in allergic reactions and the regulation of host-pathogen interactions. The distribution, morphology and biochemical composition of mast cells has been studied in detail in vitro and on tissue sections both at the light microscopic and ultrastructural level. More recently, the development of fluorescent reporter strains and intravital imaging modalities has enabled first glimpses of the real-time behavior of mast cells in situ. In this review, we describe commonly used imaging approaches to study mast cells in cell culture as well as within normal and diseased tissues. We further describe the interrogation of mast cell function via imaging by providing a detailed description of mast cell-nerve plexus interactions in the intestinal tract. Together, visualizing mast cells has expanded our view of these cells in health and disease.

KEYWORDS

cell lineages and subsets, enteric nervous system, ex vivo imaging techniques, in vivo imaging techniques, mast cells/basophils, neuro-immune-signaling

1 | INTRODUCTION

Mast cells are versatile cells with diverse functions in both homeostasis and pathological conditions. Their roles in type I allergic reactions are well established,¹ and their crucial involvement in host-pathogen interactions is increasingly appreciated.^{2,3} While mast cells are present in most tissues, they are predominantly localized at sites contacting the environment such as the skin, mucous membranes, airways and intestine; therefore, mast cells are considered sentinel cells for environmental insults.^{1,4} Anatomically, mast cells localize in close proximity to blood vessels, lymphatics as well as nerve fibres,³ which correlates with their functions, including the immediate release of preformed pro-inflammatory mediators upon encounter of stimulating agents and effector immune cell recruitment (Figures 1 and 2).

Developmentally, mast cells are hematopoietic cells and their numbers within a tissue are maintained by a combination of recruitment and maturation of committed precursors from the bone

marrow¹⁻³ as well as self-renewal of tissue-resident mature mast cells under certain settings.⁵ While many features such as ontogeny, tissue residence and similar morphology with prominent cytoplasmic granules are common mast cell characteristics, they are in fact a heterogeneous cell population. Based on their differential protease content and staining properties, mast cells have been categorized into mucosal and connective tissue mast cells. Mucosal mast cells found in the airway or intestinal mucosae are potent producers of leukotrienes LTC₄ post IgE-mediated activation⁶ and do not respond to the stabilizing activities of disodium cromoglycate.⁷ In terms of the protease content, mucosal mast cell granules contain chymases (mMCP-1 and mMCP-2) coupled to chondroitin sulfate chains of serglycin proteoglycans.⁸ Connective tissue mast cells found in the skin and peritoneal cavity, primarily produce prostaglandin D₂ and are stabilized effectively by disodium cromoglycate. Their granules contain chymases (mMCP-4), tryptases (mMCP-5 and mMCP-6), and carboxypeptidase A (mCPA) that are coupled to heparin chains of serglycin proteoglycans.⁸ Analogous to this classification in the mouse, human mast cells can be divided into subsets known as MC^T and MC^{TC}.⁹ MC^T is the mucosal site prevalent mast cell subset, which derives its name based on its protease content (tryptase). The

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Schemann and Weninger are Joint senior authors.

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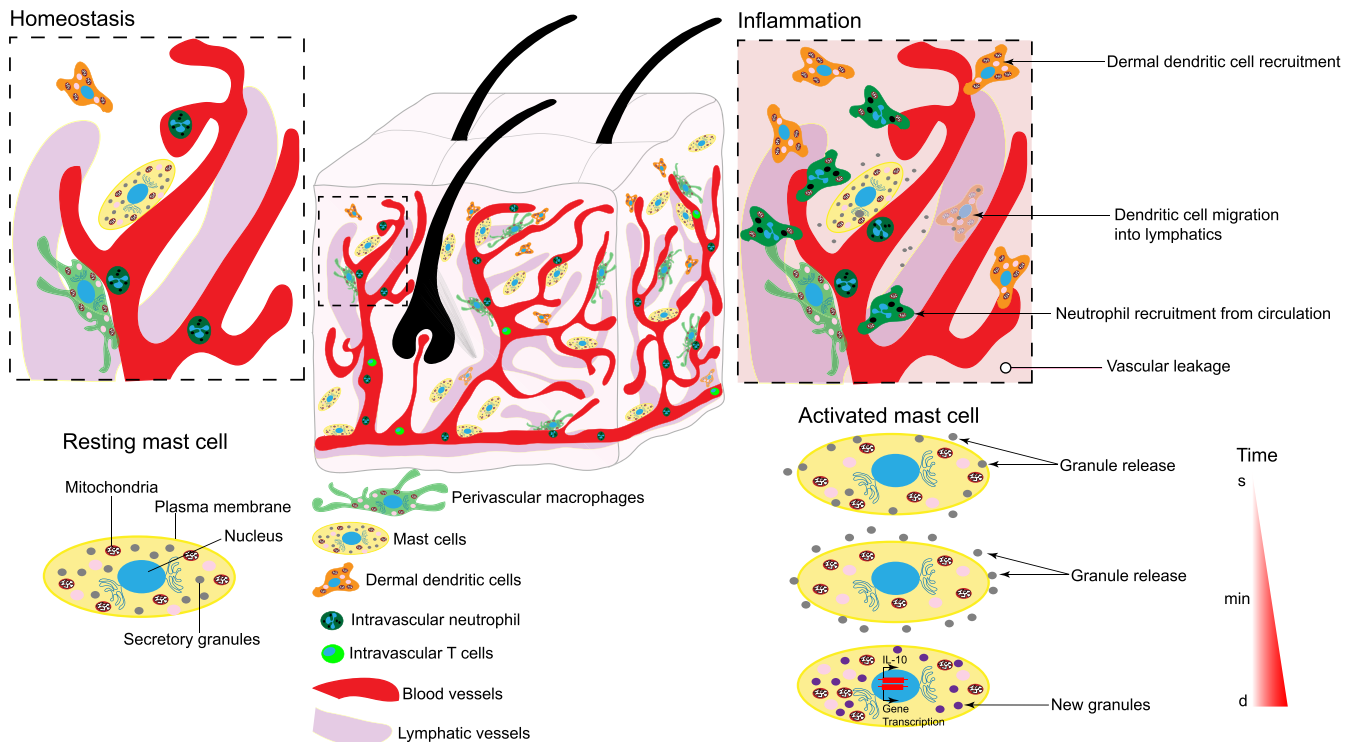


FIGURE 1 Mast cells within the skin. Schematic representation highlighting the localization of mast cells within the cutaneous tissue under homeostasis or during inflammation. Post-activation, mast cells play a crucial role in cutaneous inflammation, alter immune cell recruitment and dermal dendritic cell migration to draining lymph nodes. s, seconds; min, minutes; d, days [Colour figure can be viewed at wileyonlinelibrary.com]

MCTC subset, on the other hand, has granules comprised of tryptase and chymase.⁹ Nevertheless, this classification may be an oversimplification as there might be a spectrum of mast cell types underpinned by tissue heterogeneity and adaptation to a diverse range of potential challenges encountered.

Mast cells produce a myriad of cytokines, chemokines, lipid mediators and other factors (reviewed elsewhere^{10–12}). Activation of mast cells by pathogens or allergen-IgE complexes is followed by the release of these mediators, which occurs in two waves, namely immediate degranulation followed by de novo synthesis and release (Figure 1). Mast cell products, for example, TNF,¹³ histamine,¹⁴ VEGF,¹⁵ and proteases,¹⁶ act on the endothelium resulting in increased vascular permeability and edema³ and aid in the recruitment of other immune cells, for example, NK cells,¹⁷ dendritic cells (DCs)¹⁸ and T cells.¹⁹ Mast cell products also modulate the activation and antigen presentation status of DCs,²⁰ thereby supporting the initiation of adaptive immunity against invading pathogens.

While mast cells have been mainly studied as drivers of allergic reactions, it is increasingly clear that they exert important functions on their local microenvironment and regulate inflammatory responses particularly during infections (Figure 1). It is therefore essential to understand their behavior directly within the context of intact tissues. To this end, a variety of technologies have been developed over the years that facilitate the visualization of mast cells both at the light microscopic and ultrastructural level. In the first part of this review, we summarize recent developments in mast cell imaging. In the second part, we focus on a specific organ, that is, the intestinal tract, and highlight

how imaging has helped to obtain an in-depth understanding of mast cell functions in health and disease.

2 | MAST CELL IMAGING

2.1 | Histological staining of mast cells

Since the early years of pathologic investigation, mast cells have been assessed by the use of staining dyes that allow for the identification within tissues as well as the assessment of their morphology and intracellular structures. Mast cells can be identified on tissue sections using simple histochemical stains including hematoxylin & eosin (H&E), May-Grünwald Giemsa, Leder or toluidine blue or using more specific reagents such as antibodies against enzymes (tryptase or chymase) or the stem cell factor receptor c-kit (CD117) and FcεR1. On H&E stained sections, mast cells display round to oval blue nuclei with almost absent nucleoli, pink cytoplasm and very faint pink granules within the cytoplasm. Nonetheless, H&E is not a very reliable stain for studying mast cells.²¹ Romanowsky stain uses a combination of oxidized methylene blue (azure) dyes and Eosin Y (tetra-bromo fluorescein), which highlight purplish black to red-purple granules within the mast cell cytoplasm. On the other hand, May-Grünwald Giemsa stain comprises a mix of two neutral stains. The May-Grünwald stain is comprised of eosin and methylene blue, whereas Giemsa is composed of eosin and a basic metachromatic stain like azure of methylene. Mast cell highlighted with this stain exhibits dark blue cytoplasmic background with red granules.

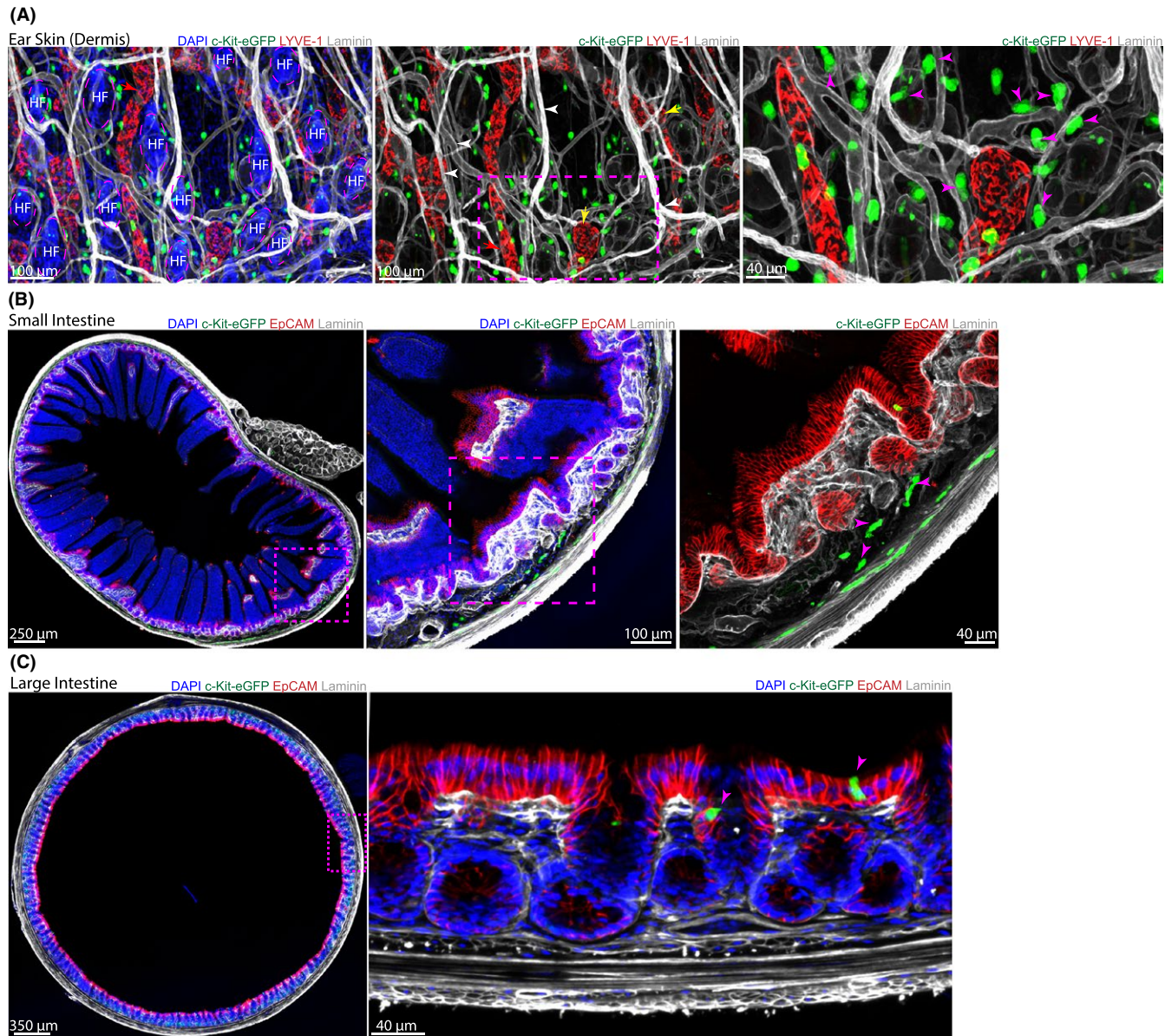


FIGURE 2 Identification of mast cells in the skin and intestines of c-kit-eGFP mice. A, Confocal images of dermal whole mounts of c-kit-eGFP mouse ear skin highlight the localization of GFP⁺ mast cells in context of various structural components. Higher magnification images (middle and right panel) highlight the intimate association of perivascular mast cells (purple arrowheads) with blood vessels (white arrowheads). Hair follicles are marked by white dotted lines (HF) and lymphatic vessels are marked by yellow arrows. B, Confocal images of small intestines highlighting the localization of GFP⁺ cells within the small intestines (purple arrowheads). C, Confocal images of large intestines highlighting the localization of GFP⁺ cells within the large intestines (purple arrowheads) [Colour figure can be viewed at wileyonlinelibrary.com]

For histochemical staining of mast cells, toluidine blue, a metachromatic stain is the method of choice. The biologically active mediators present within the mast cell granules are acidic in nature and bind basic dyes like toluidine blue. As a result, the metachromatic granules stain red-purple and the orthotropic background stains blue. Connective tissue mast cells and mucosal mast cells exhibit differing staining characteristics when stained with toluidine blue, in that the former stain readily in fixed tissues whereas the latter are sensitive to fixation. Connective tissue mast cells can be stained after fixation with 10% neutral buffer formalin, whereas mucosal mast cells can be fixed employing non-aldehyde fixatives such as Carnoy's (6 parts absolute ethanol + 3 parts chloroform + 1 part glacial acetic acid).²¹

For a more selective mast cell stain, Alcian Blue can be utilized which stains mast cells and cartilage matrix. The mast cells are easily detectable however the resolution of mast cell granules is not as sharp as with toluidine blue. Alcian blue is usually used with a counterstain like Safranin Orange or Mayer's brazalum, which stains the nuclei red.

Alternative histochemical methods rely on staining mast cells by exploiting cell-specific catalytic enzymes. The Leder stain utilizes mast cell esterase known as chloroacetate esterase to hydrolyze ester linkages in naphthol AS-D chloroacetate in the presence of diazonium salt leading to the generation of free naphthol compounds. The free naphthol then combines with diazonium salts at the site of enzymatic activity thus giving rise to strong positive red cytoplasmic staining.²²

Berberine sulfate fluorescence can also be employed as a marker for studying the secretory activity in mast cells.²³ Berberine is a cationic dye, which is weakly fluorescent and forms a strongly fluorescent complex with heparin and can thus be employed for cytofluorometric measurements of heparin within mast cells or mast cell granules. Also, fluorescently labeled avidin can be employed to highlight mast cell granules, wherein the presence of negatively charged proteoglycans within the granules allows for successful labeling of mast cells *in vivo*.²⁴

2.2 | Elucidation of mast cell ultrastructure using electron microscopic imaging modalities

Although decades of histochemical staining have been crucial for identifying the role of mast cells and their secretory granules (SG) under various pathological conditions, this methodology is inept at providing insights into granule secretion at a subcellular resolution. Therefore, electron microscopy has been employed to investigate the cellular and subcellular structure of mast cells in the steady state and post-activation. Although species, tissue, and preparation based variation in mast cell ultrastructure has been reported, for this review, we will focus on the most commonly observed structural changes in mast cell ultrastructure post-activation.

Under homeostasis, rodent mast cells display plasma membranes with finger-like projections.²⁵⁻²⁷ They often exhibit irregularly shaped nuclei with the cytoplasm being dominated by the presence of intracellular granules. These granules are separated from each other as well as the cytoplasm by the perigranular membrane. Interspersed between the granules, subcellular organelles like mitochondria, endoplasmic reticulum or Golgi apparatus can be identified.²⁸ The intra-granular structures consist of two components—fine particles and lamellar/scroll-like structures, wherein the composition of these structures varies between mast cells within different organs.^{28,29} The granular content is segregated from the perigranular membrane by an electron-lucent space.^{25,27,28,30} Several studies have highlighted the relationship between electron-opaque granular content and the perigranular membrane.²⁵ In preparations of isolated granules wherein the perigranular membranes had ruptured, the granules still retained histamine suggesting that the perigranular membrane is not a barrier to histamine release.³¹ However, addition of monovalent ions resulted in the release of histamine from isolated granules highlighting that the perigranular membrane forms an ionic insulator between the granular content and extracellular milieu or cytoplasm.²⁵

The release of prestored granules underpins both physiological and pathological roles of mast cells, thus their structure and morphology has been studied extensively during degranulation using histamine-releasing agents.^{30,32,33} Activated mast cells contain both unaltered and altered granules wherein the latter appear to be less dense, separated from their perigranular membrane, and composed of fine particles aligned as microfilaments. Post-activation with polymyxin B sulfate, mast cells show rapid and time-dependent alteration in their granule composition and structure.³⁰ A few seconds after

polymyxin B sulfate exposure, the peripheral granules are found to be associated with the plasma membrane with occasional granules showing externalization. Interaction of granules with extracellular medium results in marked structural alteration from being homogeneously electron-opaque to a diffuse fibrous meshwork. Plasma membrane fusion with the perigranular membrane as well as pore formation can also be visualized. Within minutes, most granules show altered structure,^{25,30} whereby a large number of intergranular fusions are observed leading to the formation of an extensive cytoplasmic channel along the cell periphery that opens up to the surrounding microenvironment.^{25,32} Nonetheless, other subcellular organelles like mitochondria, microtubules, centrioles, and microvesicles remain unaltered.³⁰

The response to various degranulation agents varies across mast cell types. Chemical mediators such as polymyxin B sulfate, compound 48/80 and bee venom produce similar morphological changes^{32,34,35} in skin, subcutis, and peritoneal rat mast cells. However, chemical agents like *n*-decylamine result in a change where both plasma and perigranular membranes become disintegrated with the random distribution of less dense granules throughout the cytoplasm.³² At the opposite end of this spectrum, gut mucosal mast cells are unaffected when treated with compound 48/80, suggesting functional differences between mast cells isolated from different sites.³²

Freeze-fracture electron microscopy (FFEM) shows that in unstimulated mast cells, granules appear as bulges with evenly distributed intramembranous particles within the boundary of the bulges.³⁶ This mirrors the presence of homogeneously electron-dense granules observed with transmission electron microscopy (TEM). The previously described pore formation is also observed in FFEM images of mast cells treated with polymyxin B sulfate; these pores open into large channels corresponding to the cytoplasmic matrix in mast cells.³⁶ In addition, intramembranous particles become concentrated at the base of the bulges instead and are detected near the lining of the membrane of the channel, conferring a clearer indication of granular release.³⁶

Besides establishing the morphology of activated mast cells and as an auxiliary tool to ascertain the involvement of mast cell secretion in disease states, electron microscopy has also been employed in cell-signaling studies. For instance, TEM was used to determine the spatial distribution of the components involved in mast cell signaling. The cluster patterns of the high-affinity IgE receptor (FcεRI) involved in degranulation, and two of its associated proteins, Lyn and Syk were determined using 5 or 10-nm gold particles on membrane sheets of RBL-2H3 cells.³⁷ A distinct localization pattern of Lyn and Syk exists with regard to FcεRI, which prominently changes in activated mast cells that might be driven by their respective functions down the signaling pathway.³⁷

2.3 | Atomic force microscopy

Mast cells display profound changes in membrane morphology post-activation. Although electron microscopy has been crucial in cellular structure characterization, some limitations still

exist. Electron microscopy entails sample dehydration and imaging under vacuum thus potentially compromising fine membrane structures. It also lacks 3D information crucial for the thorough investigation of structural features. Atomic force microscopy (AFM) offers a high-resolution, membrane structure characterization approach that can be employed in complementation with electron microscopy. Zink and co-workers carried out AFM imaging of bone marrow-derived mast cells (BMMCs) employing the “fix-and-look” method. They generated high-resolution 3D-structural information of BMMC membrane morphology in resting stage as well as under various perturbed conditions like dye uptake, immobilization and activation. This approach revealed well-known structural features such as ridges and microvilli along with new morphologies and nanoscopic features.³⁸ For example, post-exposure to a poly-L-lysine coated surface, crater formation on the surface of BMMC membrane was visualized and characterized. High-resolution imaging of lamellopodia and filopodia was carried out from which the movement directions were elucidated.³⁸ This study demonstrated that the “fix-and-look” approach followed by AFM imaging could provide a useful tool for carrying out the membrane structure characterization of hydrated cells with preserved structural integrity thus generating quantitative, high-resolution 3D information of cells.

2.4 | Genetic models to visualize mast cells in vivo

Histological and ultrastructural studies provide valuable insight into alterations in mast cell numbers and ultrastructure under various pathological conditions. Nonetheless, these methodologies are not amenable to studying mast cell function or their dynamic behavior. To this end, a variety of genetic mouse models have been generated (reviewed in detail in³⁹).

To image mast cells in vivo, c-Kit-eGFP mice can be employed.⁴⁰ This transgenic reporter strain harbors a BAC (bacterial artificial chromosome) reporter construct containing a 60 kb *Kit* coding sequence and 200 kb upstream sequences that specifically drive GFP expression in mast cells.⁴¹ Utilizing c-Kit-eGFP mice, Tong et al⁴⁰ carried out comparative enumeration of mast cells in ear, back, foot-pad and tail skin and found that mast cells were most abundant within the superficial dermis of ear skin. This correlated with increased edema formation in ear as compared to back skin in the passive cutaneous anaphylaxis model.

Livet and co-workers^{42,43} designed a Cre-Lox based Brainbow strategy to highlight neuronal structures in the central nervous system. The Brainbow approach entails labeling cells with distinct fluorescent proteins using a single transgene. The Cre-Lox-mediated DNA excision or inversion dependent on the orientation of lox sites triggers stochastic expression of multiple fluorescent proteins in a given cellular population. Employing Brainbow transgenic reporter mice, Roediger et al⁴⁴ showed that skin mast cells expressed high levels of the transgene. This was further confirmed by crossing the Brainbow mice with c-Kit-eGFP mice demonstrating co-expression of RFP and eGFP by mast cells.

Utilizing the BAC transgenic approach, Scholten et al⁴⁵ developed a Cre recombinase expressing transgenic mouse line wherein Cre was expressed under the control of the *Mcpt-5* promoter. When crossed to the Cre excision reporter line ROSA-26-EYFP,⁴⁶ it gave rise to *Mcpt5-CreROSA26-EYFP* double transgenic animals,⁴⁵ in which YFP is expressed in mast cells within peritoneal cavity and skin with minimal reporter gene expression observed outside the mast cell compartment.⁴⁵ This line has also been exploited for visualizing mast cell responses using intravital imaging approaches (see below).

2.5 | Intravital multiphoton microscopy of mast cells

Recent advances in the field of intravital multiphoton microscopy^{47,48} have made feasible the imaging of the cellular dynamics, migratory behavior and interactions of immune cells. Facilitated by advances in genetically engineered reporters and molecular probes, this approach has been instrumental in delineating the immune cell functions under homeostasis or during pathological conditions.^{47–52} Whilst intravital microscopy can also be carried out using spinning disk confocal microscopes, multiphoton-imaging modality remains the method of choice. This is due to the fact that the 2-photon excitation is restricted to a single focal plane resulting in reduced phototoxicity and photobleaching, enabling users to carry out deep tissue imaging for extended periods of time (albeit at slightly lower resolution than single photon confocal microscopes).

Although mast cells can be visualized in living tissues utilizing the endogenous tryptophan fluorescence,⁵³ mast cell-specific reporter strains as well as various dye based methodologies to label mast cell granules have facilitated further understanding of mast cell function in vivo.

In the case of type I allergies, the release of SG is dependent on the allergen-mediated clustering of IgE on the mast cell surface. It was assumed that mast cells acquire IgE from systemic circulation through passive diffusion. However, a recent study has highlighted that peritoneal mast cells but not dermal mast cells are uniformly labeled by intravenously injected fluorochrome-labeled IgE antibodies, suggesting that dermal mast cells take up antibodies by alternative means.⁵⁴ Employing intravital imaging, perivascular dermal mast cells were found to extend dendrites into the lumen of dermal venules thereby gaining direct access to circulating antibody in the plasma. These cells displayed dynamic probing of the vascular lumen or serial interactions with vessel wall/lumen utilizing cell body or cellular projections. Most perivascular mast cells displaying this behavior were found to reside along blood vessels with relatively lower coverage of smooth muscle actin consistent with capillaries and smaller venules. Uptake of intravascular IgE did not result in mast cell degranulation, and pharmacological blocking of mast cell degranulation via H1 and H2 histamine receptors did not alter IgE uptake suggesting that intravascular scanning behavior and IgE uptake represents a constitutive behavior independent of the activation status of mast cells. Similar intravascular scanning behavior has also been reported for mast cells in the trachea.⁵⁵ The authors utilized fluorochrome-labeled IgE (generated against

ovalbumin) to image mast cells in live slice preparations of trachea from CD11c-YFP mice⁵⁵, wherein DCs are labeled by endogenous expression of YFP.⁵⁶ Similar to the previous report, labeling of mast cells with fluorochrome-labeled IgE did not induce activation or calcium signaling. Within the trachea, DCs were predominantly localized closer to the luminal surface of the epithelium whereas mast cells were present throughout. Very few mast cells were found to interact with DCs under homeostasis or post-antigen challenge. Live imaging highlighted the sessile nature of DCs and mast cells in the absence or presence of antigen challenge, although the number of DCs increased post-antigen challenge. Within the trachea, DCs were found to extend dendritic projections under the airway epithelium but not into the airway lumen. Mast cells were found to probe the vasculature with the probing frequency increasing post-allergen challenge.⁵⁵ Whether intraluminal dendrite scanning employed by mast cells has other functional roles needs to be evaluated further.

Within the skin, mast cells are distributed throughout the dermis with significant regional variations observed between different cutaneous sites.^{40,57,58} Using double transgenic Mcpt5-CreROSA26-EYFP mice coupled with intravital microscopy, Dudeck et al visualized mast cells and blood vessels in the ear skin of control and DNFB-treated mice.⁵⁹ Similar to previous studies,^{40,54} untreated ear skin showed the presence of immobile mast cells predominantly along the blood vessels. DNFB challenge led to a dramatic increase in the diameter of the blood vessels as well as an increase in vascular permeability presumably due to the release of mast cell preformed mediators and their effects on the endothelium.⁵⁹ This was accompanied by the alteration of mast cell morphology from branched/spindle like to more globular in appearance.⁵⁹

Using fluorochrome-labeled avidin (sulforhodamine 101-coupled Avidin, Av.SRho), Galli and colleagues²⁴ visualized the release of SG from mast cells in a murine model for contact hypersensitivity. The presence of negatively charged proteoglycans along with low pH resulted in long-term labeling of the granules with the avidin probe. This methodology was able to successfully label ~90% of Mcpt5-EYFP cells in the dermis and did not alter mast cell degranulation. Similar to the studies detailed above,^{40,59} intravital imaging of mast cell degranulation highlighted the extravasation of vascular dyes. Interestingly, the vascular leakage occurred before any detectable release of labeled mast cell granules, probably mediated by histamine release after activation. Post degranulation, few of the mast granules appeared to be transported further into the tissue, akin to a previous report⁶⁰ where intact mast cell granules traveled to draining lymph nodes. Fluorescent avidin based labeling of mast cells in the IL-10 GFP reporter strain showed the presence of predominantly Av.SRho⁺GFP⁺ mast cells under homeostasis. Post-challenge, mast cells were identified as a source of IL-10 within the activated tissue with approximately 40% of avidin labeled mast cells expressing GFP.²⁴ However, within 2 days, other immune cells contributed to the production of IL-10 with mast cells only constituting approximately 10%-15% of GFP⁺ cells within the inflamed tissue. The presence of mast cells and IL-10 was intimately linked to the suppression of inflammation during severe contact hypersensitivity.

The release of mast cell-derived mediators either acutely via pre-stored granules or post-transcription has long been known to modulate innate and adaptive immune response.^{2,3} The recent identification of innate lymphoid cells (ILC) has added a new cellular player implicated in modulating immune responses in close coordination with mast cells. Using Brainbow mice, intravital imaging of the skin highlighted ILC2 mediated scanning of cutaneous mast cells under homeostasis.⁴⁴ In vitro analysis identified IL-13 (released by ILC2 under various conditions) to suppress IgE-dependent cytokine release by mast cells. This suppression was specific for IL-13 as co-incubation of mast cells with IL-9 led to enhanced IL-6 and TNF- α production.⁴⁴ In helminth infected murine gut, the release of mast cell-derived IL-33 has been shown to contribute to ILC2 recruitment and activation, resulting in IL-13 production by ILC2 and amplification of type-2 immune response.⁶¹ A similar interplay between mast cells-ILC2 and TH9 pathways has been implicated in promoting inflammation during cystic fibrosis.⁶² These studies provide a glimpse into the communications between mast cells and ILCs under homeostasis and during pathological inflammation.

Other than modulating the immune response, mast cells have garnered attention for their crucial role in pregnancy. Early on, the presence of an increased number of mast cells is important for implantation, placentation and fetal growth, for example, the poor implantation and lower birth rate observed in mast cell-deficient C57BL/6-Kit^{W-sh/W-sh} mice could be rescued by systemic or local transfer of BMMCs.⁶³ These effects are mediated by mast cell-derived factors that alter the surrounding tissue microenvironment, especially spiral artery remodeling.⁶³ Intravital imaging of uterine tissue in vivo highlighted an increase in connective tissue mast cell density during the estrus phase. These mast cells localized in close proximity to blood vessels and were maintained during pregnancy albeit in lower numbers than the estrus phase.⁶⁴ During late pregnancy, inadvertent activation of mast cells has been implicated in preterm delivery.⁶⁵ Although recent studies highlight the crucial role of mast cells during transplantation and pregnancy,^{63,65} how these cells alter local structural and immunological microenvironment at the evolving maternal-embryonic interface needs further investigation. Therefore, the use of intravital imaging holds great potential in understanding the dynamic role of mast cells in pregnancy.

2.6 | pH sensors for studying degranulation in vitro and in vivo

Mast cell degranulation has been studied extensively employing histamine and β -hexosaminidase release measurements, amperometry, immunodetection of plasma membrane-associated proteins as well as electron microscopy.^{66,67} However, to visualize the degranulation of mast cells in real time at the single cell level, Horiguchi and coworkers⁶⁶ developed a fluorescent protein-based indicator of degranulation termed as immune-pHluorin (impH) which is pHluorin fused to VAMP-8. pHluorin is non-fluorescent at pH <6 and turns fluorescent at neutral pH. To test the potential of impH in vivo, the ear skin of mast cell-deficient C57BL/6-Kit^{W-sh/W-sh} mice was reconstituted with impH- expressing BMMCs from either wildtype or FceR1^{-/-} mice.

To induce cutaneous anaphylaxis, the transferred BMMCs were intradermally sensitized with TNP-specific IgE. The mice were subsequently challenged with TNP-OVA. Intravital microscopy revealed the presence of green fluorescent patches in wildtype, but not FcεR1^{-/-} BMMCs 10 minutes post intravenous administration of TNP-OVA.⁶⁶ This suggests that impH is a useful tool for visualizing mast cell degranulation in vivo.

2.7 | Negative contrast imaging

To directly visualize the biology of mast cell SG in vitro, Tanaka and Takakuwa employed negative contrast imaging. In this imaging modality, soluble GFP is expressed in the cytoplasm of the mast cells thereby highlighting SG by virtue of exclusion of GFP.⁶⁸ When employed in combination with other fluorescent probes, it potentiated visualization of SG-SG fusion in PMA-activated RBL-2H3 cells.⁶⁸ The authors reasoned that this modality could be useful for studying SG biogenesis, maintenance, regulation, and recycling thus assisting in finding better therapeutic targets for allergic disorders.

2.8 | Other approaches for mast cell tracking in vivo

To better understand the role of mast cells in cancer progression, Oh and co-workers⁶⁹ employed a non-invasive imaging modality by tagging murine mast cells (MC-9) with enhanced firefly luciferase (efluc). The authors visualized MC-9 efflux cell migration to LLC (Lewis lung cell carcinoma) lesions. In vivo optical imaging along with the bio-distribution study employing ¹⁸F-FDG (¹⁸F-fluorodeoxyglucose) demonstrated enhanced tumor growth and elevated glucose uptake presumably associated with the mast cell migration to LLC lesions.⁶⁹

In addition, MRI (magnetic resonance imaging) has been carried out to image mast cell tumors in canines.⁷⁰ MRI has been shown to be a useful tool in clinical staging and presurgical evaluation of cutaneous mast cell tumors.⁷⁰ Ultrasonography has been utilized to study the features of feline mast cell tumors.⁷¹ FDG-PET (positron emission tomography) has been carried out in patients with systemic mastocytosis but was shown to be not useful for staging or follow-up of aggressive systemic mastocytosis.⁷²

3 | MAST CELL-NERVE SIGNALING IN THE GUT

The gastrointestinal tract is a highly complex integrated organ system. It contains two remarkable networks—one is the enteric nervous system (ENS) and the other one is the enteric immune system (EIS). The ENS consists of interconnected neuronal plexuses with close associations with immune cells, such as macrophages, mast cells, and lymphocytes, which are particularly abundant in the lamina propria. There are two main nerve plexuses in the ENS: the myenteric plexus is located between the muscle layers and controls motility whereas the submucous plexus is located just beneath the mucosa and regulates epithelial functions. Together with the extrinsic nerve supply of vagal

and spinal origin, the ENS and EIS are believed to form a complete sensory web, regulating appropriate epithelial (secretion and absorption) and muscle (motility) responses to the luminal environment and changes in the milieu of the gut wall.^{73,74}

Immune cells such as mast cells are active partners in regulating gut function. These interactions are also involved in the pathophysiology of inflammatory and functional bowel diseases.⁷⁵ Over the past years it has become increasingly evident that mast cells play a significant role in gut physiology. Interaction between nerves and mast cells has been extensively studied in both health and disease. This communication is facilitated by their close proximity, expression of complementary receptors on mast cell and nerves and receptor-mediated activation of intracellular signaling pathways.⁷⁶ During inflammation, this interaction is amplified by the influx of immune cells, significantly altering the microenvironment.⁷⁷ In this context, the specific neurotransmitters and mediators released by neurons and/or mast cells may either have a pro- or anti-inflammatory effect.⁷⁸ Persistent inflammation in the GI tract has been suggested to result in neuroimmune plasticity.

In this section, we describe the approaches used to monitor interaction between mast cells and nerves within the gut in both animal and human tissue with an emphasis on functional imaging of nerve and mast cell activity (Figure 4). It is not our intention to provide a comprehensive overview of mast cell-nerve interactions but we rather focus on mast cell-nerve crosstalk in the gut (Figure 4).

3.1 | Nerve and mast cell stimulation- feedback loop

Enteric nerves express an array of receptors, complementary for mediators released by mast cell degranulation. Similarly, neuropeptides released by nerves can modulate mast cell activation, and hence degranulation. Most of the studies on nerve-MC signaling focused on experiments in cell culture and rodent animal models.⁷⁹⁻⁸¹ Under normal conditions, and even more so during inflammation, neurons generate impulses, resulting in the release of adenosine triphosphate, vasoactive intestinal peptide (VIP), substance P (SP), and calcitonin gene-related peptide (CGRP), all of which are believed to contribute to nerve triggered mast cell activation.⁸²

Crosslinking of the FcεRI via IgE-antigen complexes leads to human intestinal mast cell activation and subsequent degranulation.⁸³ The non-IgE-mediated activation of mast cells can be triggered by a variety of different stimuli, including, IgG, complement component, toll-like receptor ligands, neuropeptides, cytokines, chemokines, and other inflammatory products⁸² (Figure 4). Upon degranulation, there is release of preformed mediators stored in SG or de novo synthesis of mediators.

Some key mast cell mediators that are released are histamine, serine proteases, interleukin-1β, leukotrienes, prostaglandins and in rodent but not human intestine serotonin⁸² (Figure 4). Mediators released by degranulation can evoke an inflammatory response by acting directly on surrounding tissue or indirectly through effector cells, such as epithelial cells, residential macrophages, and enteric nerves.

Mediators released by mast cells are crucial for neuroimmune communication, and hence have been used as an effective tool to measure the activity and degranulation of mast cells and subsequently

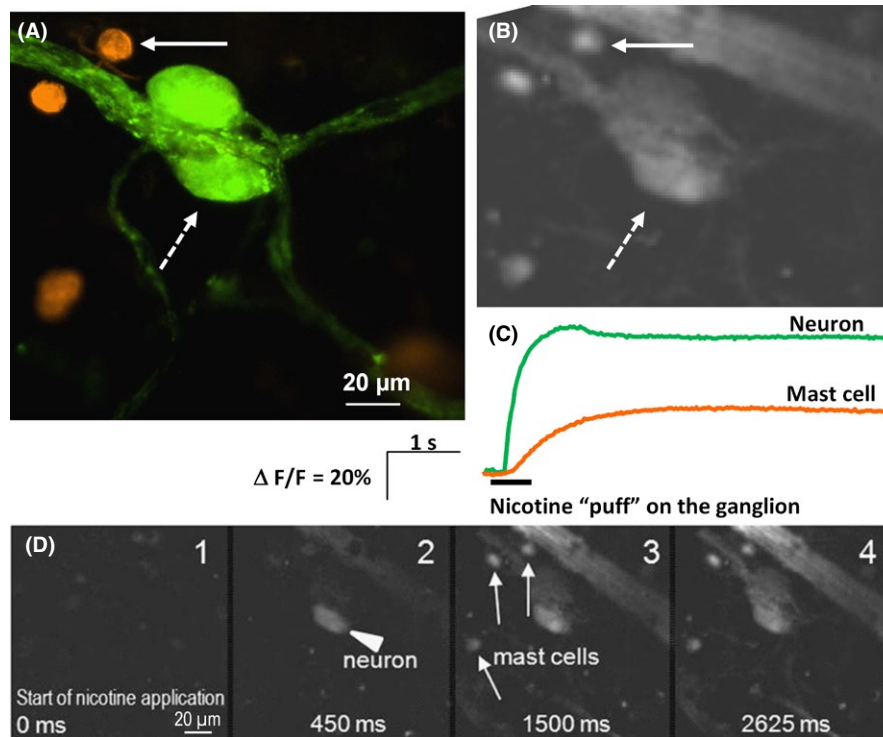


FIGURE 3 $[Ca^{+2}]_i$ transients in mast cells in response to selective nerve activation by nicotine in human submucous plexus preparations (A–D). In (A) the post-experiment immunohistochemical labeling demonstrates nerve cell bodies and fibers (PGP9.5 in green) and mast cells (c-kit in orange). The neurons in the small ganglion were activated by a brief pressure microinjection (“puff”) of nicotine which only activates neurons. Trace of the responding neuron (marked by the dotted arrow) is shown in C (green trace). All 3 mast cells to the left responded with $[Ca^{+2}]_i$ increase (one is marked by solid arrow in A and B). B shows one image taken at the peak of the $[Ca^{+2}]_i$ response during the actual experiment. The full response of the marked mast cell is shown in C (orange trace). The bar below the trace indicates the 500 ms application of nicotine. D shows 4 frames (1–4) which illustrate the sequence of the mast cell response after nerve stimulation by nicotine. The $[Ca^{+2}]_i$ response first started in the neurons (arrowhead frame 2) followed by the $[Ca^{+2}]_i$ response in the mast cells (frame 3 and 4, all 3 mast cells are marked by solid arrows in frame 3) [Colour figure can be viewed at wileyonlinelibrary.com]

their communication with nerves in normal and diseased conditions. Most of the evidence for functional neuroimmune communication was previously only demonstrated by animal studies. However, in recent years, there have been significant advances, reporting the effect of these mediators on human tissue.^{84–98}

3.2 | Mast cell and enteric nerves: Next-door neighbors

Initial evidence for interaction between nerves and mast cells was derived from their close anatomical proximity in various organ systems, including the gut. These findings provided an anatomical basis for interaction both in physiology and pathophysiology of the GI tract. In healthy individuals, mast cells are primarily located within the lamina propria, followed by the submucosa and fewer in the muscle layer and serosa.^{76,82} In the human GI tract, mast cells in the lamina propria lie in close proximity to nerves (Figure 4). Further anatomical analysis reported that about 90% of intestinal MCs are located at least within 2 μm to nerves.^{99–101} It has also been reported that a small percentage of mast cells also share synaptic like contacts with nerve fibres.¹⁰² These synaptic like contacts can also be induced, enhanced or blocked, using specific adhesion molecules,

such as cell adhesion molecule 1 and nectin 3 localized to the nerve.¹⁰³ More commonly, mast cells are located close to nerves without forming synaptic like contacts, which would require volume transmission for interactions. It has been suggested that mast cells release neurotrophic factors and cytokines, which promote elongation of nerves in close proximity to mast cells. This microanatomic association can promote interaction in maintaining mucosal homeostasis. A lot of emphasis has been given to the increased counts of mast cells present in the colonic and ileal mucosa of patients with irritable bowel syndrome (IBS).¹⁰⁰

It has been demonstrated that upon activation, mast cells release mediators, resulting in increased neuronal excitability leading to visceral hypersensitivity and abnormal gut motor function. The increase in mast cell count significantly correlates with the frequency and severity of abdominal discomfort, suggesting that increased infiltration of mast cells in close proximity to mucosal nerves may contribute to hypersensitivity in IBS patients.¹⁰⁰ In line with this, mast cells have been found in close apposition with nerves containing SP and CGRP in humans and rodents gastrointestinal tract.⁷⁶ However, according to anatomical data, these interactions may be more predominant with enteric neurons, because in human intestine, fewer mast cells are in close proximity to extrinsic afferents expressing transient receptor potential vanilloid 1 channels.⁷⁶

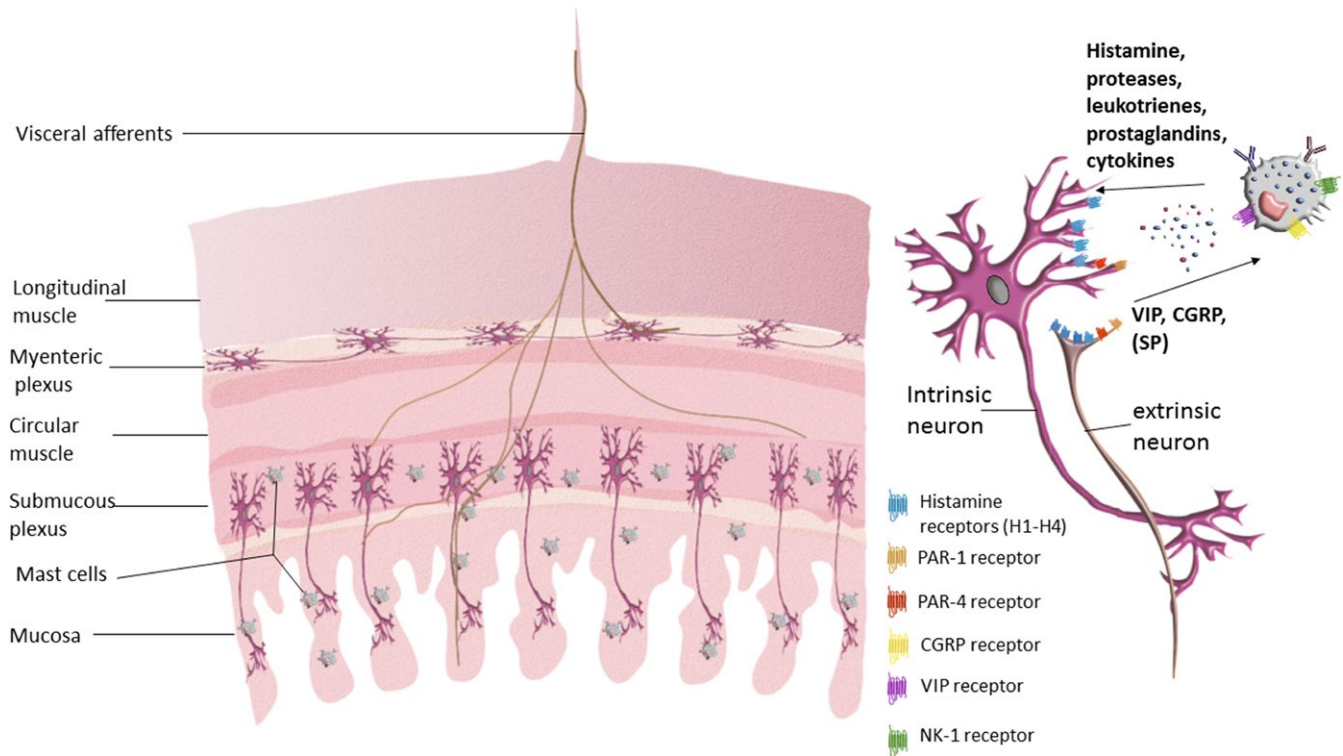


FIGURE 4 Mast cell nerve interactions in the gut wall. Schematic drawing of the intestinal layers illustrating that mast cell-nerve signaling is mostly present in the lamina propria in the submucous and mucosal layer. The illustration on the right zooms into an association between mast cells and intrinsic (enteric) nerves or terminal endings of visceral afferents (vagal or spinal). The mast cell mediators affecting nerve activity and the neurotransmitters with influence on mast cell activity as well as the involved receptors are those relevant in the human intestine. SP is in parenthesis as the functional role in gut tissue has not been unequivocally demonstrated [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Functional evidence for neuroimmune interaction

Functional evidence of interaction between mast cell and nerves is mainly derived from electrophysiological recordings in cell culture models. Imaging techniques using fast optical recordings are powerful tools to study neuronal activity within the gut and to detect direct signaling between nerves and mast cells. In recent years, we have advanced imaging techniques to record neural activity in cell culture, as well as in isolated intact preparations of both rodent and human tissue.¹⁰⁴⁻¹⁰⁷ These microscopic imaging modalities have provided invaluable information about several aspects of neuronal signaling.¹⁰⁷⁻¹⁰⁹ In particular, we uncovered various electrical and synaptic properties of neurons in response to chemical and mechanical stimuli.^{105,107,110,111} In addition, a lot of advances have been made in understanding the interaction of enteric neurons with other cell types, such as mast cells.^{76,82}

Fluorescent imaging, in particular, voltage-sensitive dye (VSD) imaging has provided many significant advances in neuronal biophysics and circuit function. VSDs allow the detection of neuronal activity at a single cell level. The potentiometric dye best-suited for investigating enteric neurons is di-8-ANEPPS (di-8-butyl-amino-naphthyl-ethylene-pyridinium-propyl-sulfonate)¹⁰⁴ which enables non-invasive observations of neuronal activity. Microinjection of di-8-ANEPPS into the ganglia allows the dye to be incorporated into the lipid bilayer of the cell membrane, resulting in strong fluorescence^{105,107} without changing

the electrophysiological properties of individual nerve cells.¹⁰⁵ Since the dye incorporates into the membrane, revealing the outline of individual cell bodies, it is possible to record activity from hundreds to several thousand neurons in the gut wall, while still keeping track of the activity of individual neurons. Similarly, calcium imaging has also been extensively utilized to unravel signaling pathways associated with ENS stimulation.¹⁰⁶ In contrast to VSD imaging, calcium imaging also allows the simultaneous measurement of several cell types with and without fast Na channels, hence allowing the measurement of interaction between spiking and non-spiking cells.

Using imaging techniques, we have obtained a lot of information regarding mast cell and nerve activation, the effect of individual mast cell mediators on neurons and neurotransmitters on mast cells, as well as the receptor mechanism of these responses. Since the mast cell to nerve signaling is predominantly through the release of histamine and tryptase, these two mediators have been extensively used to identify their mode of action.

3.4 | Mast cell to nerve signaling through histamine receptors

Histamine is identified as a major mediator in neuroimmune signaling in the gut. The effect of histamine on neuronal responses has been studied in animal and human preparations. Increased expression of H1 and H2 receptors has been reported in biopsies of patients with

IBS and food allergy.¹¹² Histamine receptors are therefore a promising target for gut dysfunction, in particular IBS.¹¹³ Using VSD imaging, we confirmed previous results in guinea pig ENS demonstrating excitatory H1 and H2 receptors and presynaptic inhibitory H3 receptors.⁹⁰ Interestingly, similar study in human tissue preparations demonstrated that histamine excites human enteric neurones and this effect involves all four histamine receptors (H1, H2, H3 and H4).⁹⁰ Contrary to the H3 mediated inhibition observed in guinea pig tissue, activation of H3 in humans results in neuronal excitation. Similar discrepancy in rodent and human studies was also observed with proteases and their effect on enteric or sensory nerves. The neural action of proteases is primarily through protease-activated receptors (PAR) 2 in rodents,¹¹⁴ but mainly through PAR-1 in human intestines.⁹⁵ These differences necessitate the need for more human studies and question the translational relevance of studies performed with primary culture or rodents in humans.

3.5 | Mast cell signaling through protease activated receptors

The effect of serine proteases released by mast cells (and from intestinal bacteria) on mast cell-nerve signaling in the gut, has been studied in detail. Tryptase, a serine protease is present in all mast cell subtypes, comprising approximately 25% of their total protein content and is released readily upon degranulation.⁸² Tryptase has been shown to signal to neurons through PARs located on enteric neurons and visceral afferents. Proteases evoke nerve responses through the cleavage of the extracellular N-terminal tails of PARs to expose tethered ligand domains that bind and activate cleaved receptors, activating PAR receptors.¹¹⁵ There are four subtypes of PAR receptors reported in human intestine (PAR-1, PAR-2, PAR-3, and PAR-4). These receptors are abundantly expressed throughout the gastrointestinal tract on various cell types including neurons present in the myenteric and submucosal plexus. Proteases are significantly increased in the mucosa of IBS patients.^{96,114} Tryptase has been shown to cleave PAR-2 on primary spinal afferent neurons in rodents, which causes the release of SP, activation of the neurokinin 1 receptor, and amplification of inflammation and thermal and mechanical hyperalgesia. Thrombin predominantly activates PAR-1, PAR-3, and PAR-4, while trypsin and mast cell tryptase act on mainly on PAR-2. Nociceptive effects of tryptase and trypsin evoked in mice are reduced by serine protease inhibitors as well as by PAR-2 receptor antagonist or in PAR-2-deficient mice.¹¹⁶ These studies demonstrate the stimulatory effects of proteases, resulting in pain sensitivity through PAR receptors.

Although these studies provide a good basis for functional interaction between mast cell and nerves during *in vivo* mast cell degranulation; it was unclear whether the concentration of mediators used in these studies reflected the concentration of individual mediators used *in vitro* studies. Another aspect that wasn't accounted for in these studies was the possible synergistic effect that might be accountable for neuronal excitation in mast cell to nerve signaling. To address these issues, we assessed whether a mediator cocktail released from human intestinal mast cell activated by IgE receptor cross-linking can elicit a

neuronal response in human and guinea pig enteric neurons. Using the VSD imaging technique, we found that application of the mast cell mediator cocktail resulted in action potential discharge in 31%, 38%, or 11% neurons of guinea-pig submucous plexus, human submucous plexus, or guinea-pig myenteric plexus, respectively.⁹² This study gave an initial insight into the functional neuroimmune interaction in guinea pig and, more importantly, the human gut. This study also provided evidence for the increased responsiveness of submucous compared to myenteric neurons to the mast cell mediator cocktail. This finding seems to be the functional correlate of the increased presence of mast cells around enteric nerves in the epithelial and sub-epithelial layer, with very few present in the human myenteric plexus and muscle layers.⁷⁶

3.6 | Mast cell-nerve communication in IBS

As previously mentioned, there is strong evidence for a role of mast cells in the pathophysiology of IBS. These include increased mast cell counts in intestinal mucosa of patients with IBS compared to healthy controls.¹⁰⁰ An increase in the number of mast cells has been found in the colon, cecum, ileum, jejunum, and duodenum.^{96,117-122} In addition, there have been reports suggesting an increase in mast cell reactivity rather than an increase in density. Degranulated mast cells were more prominent in the colon of IBS patients.¹⁰⁰ These activated mast cells were found in close proximity to nerves containing 5HT and SP,¹²³ acting as an amplifying loop where neuropeptides released from nerve endings activate mast cell degranulation, releasing histamine, which further induces a release of neurotransmitters from sensory endings.

There have been several functional studies investigating the effect of mast cells on nerves in IBS. These functional studies include the use of mucosal biopsies and luminal content of the GI tract. Mucosal biopsies can be cultured *in vitro* and the resulting supernatants can facilitate measurement of neuronal responses.⁹³ Using similar approach, we demonstrated that supernatants obtained from culturing mucosal biopsies from IBS patients are more likely to activate human and guinea pig enteric neurons compared to supernatant from healthy subjects.⁹⁶ This nerve excitability is regulated by mast cell-derived mediators, including histamine and serine proteases.⁹⁶ Furthermore, neuronal responses correlate with the increased mast cell density and the histamine and protease levels found in IBS supernatant. These changes have been observed across different IBS subtypes independent of whether their predominant stool irregularity is diarrhea or constipation, suggesting that mast cell-nerve signaling is a relevant pathological factor in IBS.⁹⁶ In addition, application of IBS supernatants results in increased neuronal excitability, which is more predominant in the submucous plexus (46%) compared to the myenteric plexus (8.5%).⁸⁸

To further investigate any synergistic effect that might be involved in mast cell-nerve signaling, we prepared a cocktail of various mast cell mediators found in IBS supernatant and investigated its effect on submucous plexus neurons of biopsies obtained from IBS patients. This "IBS cocktail" contained histamine, serotonin, tryptase and TNF- α (IBS cocktail).⁸⁶ Interestingly, a decrease in the responses to these mediators was observed in biopsies from IBS patients, again independent of the IBS subtypes. The reason for this reduced activity was suggested to be a

possible desensitization to mediators, in particular proteases, constantly released by mucosal and immune cells within the gut wall of IBS patients. Even low amounts of these mediators have been shown to exert changes in neuronal activity. It has been demonstrated that this is due a synergistic effect of tryptase on the actions of histamine and serotonin, resulting in potentiation of the neuronal response.⁸⁴ The synergistic potentiation between histamine, serotonin, and tryptase in enteric neurons is PAR-2 independent and likely involves PAR-1 or PAR independent proteolytic activity.⁸⁴ This finding explains neuronal activation by a cocktail of these mediators despite their low concentrations in IBS supernatants and despite a relatively small PAR-2-mediated response in human submucous neurons. Again, it needs to be stressed that serine proteases mainly signal through PAR-1 and not PAR-2 in human enteric neurons.^{94,95}

3.7 | Bidirectional interaction in intact human tissue

The first bidirectional communication was demonstrated using an *in vitro* co-culture approach comprising of cultured murine superior cervical ganglia (SCG) and rat basophilic leukaemia (RBL) cells. It was reported that activating mast cells by anti-IgE receptor antibodies results in increased intracellular calcium concentration in SCG neurites.¹²⁴ The same group also reported the activation of mast cell through the release of SP from nerves.

Another study demonstrated bidirectional communication between mast cells and dorsal root ganglion (DRG) neurons using primary culture of murine bone marrow mast cells. Nerve to mast cell signaling was demonstrated by the application of CGRP, resulting in mast cell activation.⁸¹ Mast cell to nerve signaling was reported by stimulation of mast cells by compound 48/80, resulting in Ca^{++} increase in DRG neurons.⁸⁰ Compound 48/80 is widely used to activate and degranulate mast cells. We have contradicted this notion and demonstrated the non-specific effect of this compound.¹²⁵ We found that it evoked spike discharge and calcium transient in cultured DRGs (mouse) and enteric neurons (guinea pig). These cultures were devoid of mast cells and hence showed a direct effect of this compound on nerves. Therefore, compound 48/80 should be used with caution as the non-specific effect of this drug needs to be considered when interpreting data in model systems containing neurons.

There are differences in the neuropharmacology of receptors involved in mast cell nerve signaling between human and rodents. Studies using human intestinal mast cells cultures have also reported discrepancies in mast cell activation in response to activation by neurotransmitters.⁹¹ These differences have been attributed to the development of plasticity of cultured mast cells, possibly compromising their expression of relevant receptors. It was vital to demonstrate this interaction in human tissue. These observations suggest that the bidirectional communication between mast cells and nerves should be investigated in intact human tissue, where both cell types are exposed to their physiologic milieu. We recently demonstrated such interaction in intact human submucous plexus.⁸⁵ With the use of calcium imaging, intracellular calcium transients were simultaneously recorded from mast cells and enteric neurons (Figure 3). Using a monoclonal antibody (mAb22E7), mast cells were specifically stimulated by IgE receptor

cross-linking. Similarly, neurons were specifically activated using electrical stimulation which was followed by subsequent activation of mast cells. The Ca^{2+} signals in mast cells were completely blocked when the neurotransmission was inhibited by the nerve blocker tetrodotoxin clearly demonstrating the participation of neurons. The effects were mainly mediated by the release of the neuropeptides CGRP and VIP. Blocking specifically the CGRP1 receptor with the high-affinity antagonist BIBN4096 as well as blocking the VPAC1 receptor by PG97-269 abolished or reduced, respectively, the mast cell activation upon nerve stimulation. Interestingly and contrary to common belief, nerve to mast cell signaling was frequently observed in our human intestinal preparations, whereas mast cell to nerve signaling was very rare.⁸⁵ This study provided, for the first time, bidirectional interaction between mast cells and nerves in intact human tissue, where both cell types are exposed to their physiological environment. Using the same tools, it would be interesting to see whether this interaction is altered in diseased tissue. Especially, does the signaling from mast cells to nerves become more prominent, and if so, what are the mediators that might be involved in this increased signaling. These studies can be conducted, using the same imaging approach in inflamed gut sections.

The mast cell response patterns resemble those of neuropeptides on single mast cells in cell culture and rodent intestine.^{80,126} For example, the activation of the CGRP1 receptor on mast cells in mice resulted in a differential or piecemeal release of mediators rather than in total degranulation.¹²⁶ Piecemeal release is defined by the release of relatively small amounts involving selective secretion of only certain mediators. It is considered to play a dominant role during inflammation.¹²⁷ Interestingly, in rat intestinal mucosa, stress-induced piecemeal release was observed primarily in mast cells which were in close proximity to nerves.¹²⁸ To our surprise, we did not find a significant role for SP since the nerve mediated mast cell response remained unchanged after blockade of neurokinin 1,2 and 3 receptors. However, this agrees with the finding that only few mast cells in the human intestine express the neurokinin 1 receptor which has the highest affinity for SP.⁸¹ Even the presence of neurokinin receptors on mammalian mast cells is controversial.¹²⁹ Bischoff et al for example, showed that cultured human intestinal mast cells do not constitutively express neurokinin receptors⁹¹; however, when primed by stem cell factor or interleukin-4, they started to express neurokinin 1 receptors and responded to SP with degranulation. Thus, SP effects on mast cells may vary with their precondition, for example, inflammation. A further aspect concerning the SP action on mast cells derived from two studies on patch clamp¹³⁰ or imaging MC activity in cell culture.¹³¹ Picomolar SP concentrations triggered electrical oscillations of rat peritoneal mast cells in the absence of degranulation; only repetitive SP applications resulted in mast cell degranulation.¹³⁰ The authors conclude that their findings are the functional correlate of priming, a phenomenon that sensitizes mast cells to subsequent stimuli. It was speculated that this feature may enhance the efficacy of mast cell responses to antigen or neuropeptide challenge. Mast cells exert distinct activation and release patterns and the specific features of different stimuli may influence the responsiveness to SP.¹³¹

3.8 | Interplay between mast cells and extrinsic sensory neurons

The CNS receives specific information on the normal and inflammatory state of the bowel, enabling orchestration of timely responses to the presence of antigens.⁷⁴ The afferent innervation of the GI tract can be divided into vagal afferents and spinal afferents. Visceral afferents in the gut wall are activated by various physiological stimuli, including changes in intestinal milieu. Under pathophysiology, terminals of these afferents also respond to inflammatory mediators.

The terminals of vagal and spinal sensory neurons express receptors for multiple mast cell mediators (Figure 4).^{74,132} Consistent with this finding, various studies have shown that sensitization of sensory nerve terminals by mast cell mediators which seems related to hypersensitivity in response to intestinal distension in IBS patients.^{133,134} Tryptase activates spinal afferents through PAR-2, releasing SP, activating neurokinin 1 receptor, resulting in increased hyperalgesia in rodents.¹³⁵ Interestingly, functional signaling between mast cells and spinal afferents has been suggested to be modulated by enteric neurons.⁸¹ Afferent input results in the release of mast cells mediators, which in turn influence the behaviors of enteric neurons, and further results in activation of spinal afferents.⁸¹

An increase in the number of mast cells in colonic mucosal biopsies from IBS patients might suggest that the hypersensitivity to distension and strong muscle contractions reflects the sensitization of intramural endings of spinal mechanosensitive nerves by mediators released from mast cells. Most functional studies assessing interaction between mast cells and afferents have been primarily conducted by recording electrical responses from single neurovascular bundles in the jejunal wall using a suction electrode. A direct response of mast cell products on visceral afferents was provided by Jiang et al.¹³⁶ By conducting in vivo multiunit afferent nerve recording from mesenteric nerves, they have shown that histamine stimulates mesenteric afferents supplying the rat jejunum via histamine H1 receptors. These afferents may transmit inflammatory signals from gut mucosa to the CNS.

Compared to mast cell ENS interactions, there are fewer functional studies that have addressed interactions between mast cells and visceral afferents. Most of the studies have been conducted in animal models. In the last few years, some groups have developed an in vitro afferent nerve preparation using surgically excised freshly isolated human intestine. Signaling from immune cells to visceral afferents in the human gut are likely since inflammatory mediators result in an increased firing of afferent nerves.¹³⁷ Currently, there are no studies that have investigated the receptor mechanism involved in the afferent response to individual immune mediators in human gut tissue. Particularly, the receptor mechanism underlying responses to proteases are of significant interest.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ORCID

Shweta Tikoo  <http://orcid.org/0000-0001-5850-8075>

Rohit Jain  <http://orcid.org/0000-0002-4905-462X>

Michael Schemann  <http://orcid.org/0000-0003-1007-9843>

Wolfgang Weninger  <http://orcid.org/0000-0003-3133-8699>

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