Lack of neurokinin-1 receptor expression affects tissue mast cell numbers but not their spatial relationship with nerves

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1Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, Spring House, Pennsylvania; 2Department of Physiology, The Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma; 3Pulmonary Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts; 4Ina Sue Pelmutter Laboratory, Children’s Hospital, Harvard Medical School, Boston, Massachusetts; and 5Department of Pediatrics, The Children’s Hospital at Montefiore and Albert Einstein College of Medicine, Bronx, New York

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D’Andrea, Michael R., Marcia R. Saban, Norma P. Gerard, Barry K. Wershil, and Ricardo Saban. Lack of neurokinin-1 receptor expression affects tissue mast cell numbers but not their spatial relationship with nerves. Am J Physiol Regul Integr Comp Physiol 288: R491–R500, 2005. First published September 30, 2004; doi:10.1152/ajpregu.00452.2004.—A spatial association between mast cells and nerves has been described in both the gastrointestinal and genitourinary tracts. However, the factors that influence the anatomical relationship between mast cells and nerves have not been completely defined. It has been suggested that the high-affinity receptor for substance P [neurokinin-1 (NK1)] might modulate this interaction. We therefore assessed mast cell-nerve relationships in tissues isolated from wild-type and NK1 receptor knockout (NK1−/−) mice. We now report that, in the complete absence of NK1 receptor expression, there is a significant increase in the number of mast cells without a change in the anatomical relationship between mast cell and nerves in stomach and bladder tissues at the light microscopic level. We next determined whether transplanted mast cells would maintain their spatial distribution, number, and contact with nerve elements. For this purpose, mast cell-deficient KitW/KitW−/− mice were reconstituted with wild-type or NK1−/− bone marrow. No differences in mast cell-nerve contact were observed. These results suggest that NK1 receptor expression is important in the regulation of the number of mast cells but is not important in the interaction between mast cells and nerves. Furthermore, the interaction between mast cells and nerves is not mediated through NK1 receptor expression on the mast cell. Further studies are needed to determine the molecular pathway involved in mast cell migration and interaction with nerve elements, but the model of reconstitution of KitW/KitW−/− mice with mast cells derived from different genetically engineered mice is a useful approach to further explore these mechanisms.

Tissue mast cells, whether resident or transplanted, are in close proximity to the terminal branches of sensory and autonomic nerves (22, 47, 48) and smooth muscle cells (16–18). Spatial associations in biological systems are often indicative of functional interactions. In this context, nerve-mast cell interaction has been described in the skin and in the gastrointestinal (GI) and urinary tracts (4, 30, 33, 50). Early studies elegantly described the nonrandom spatial association of mast cells with nerves in a variety of tissues in which actual membrane-membrane contacts occur (11, 22, 32, 44, 47, 48). In the GI tract, several studies have suggested that mast cell-nerve interaction has an important homeostatic role in the regulation of gut physiology as well pathophysiology (57). Studies that have utilized mast cell-deficient KitW/KitW−/− (Kit) mice have provided data to support this nerve-mast cell interaction. For example, Kit mice subjected to enteric nerve stimulation exhibited a marked decrease in intestinal secretion compared with normal mice, and this response was fully reestablished after the mast cells were reconstituted by the administration of bone marrow-derived precursor cells (37).

Previously, our group (40) provided direct evidence that mast cells are essential for the inflammatory response and gene expression seen in experimental cystitis by comparing and contrasting the reaction of normal mice, mast cell-deficient KitW/KitW−/− (Kit) mice, and mast cell-reconstituted mice. We also showed that mast cells in the bladder are frequently in direct contact with nerve elements (22), and there was functionality associated with this connection (9, 10).

The factors that regulate mast cell-nerve interactions are still poorly understood. An increase in the local density of mast cells within inflamed tissue has been reported in asthma (25), rheumatoid arthritis (19), interstitial cystitis (51–53), and other chronic inflammatory conditions. Mast cell-nerve communication seems to be increased in cystitis (13, 35), suggesting that inflammation results in the production of mediators that modulate this connection, either by enhanced recruitment of mast cell precursors from the circulation or by the local proliferation and/or migration of resident mast cells (56). In both irritable bowel syndrome (IBS) and inflammatory bowel disease, the number of mast cells was noted to be increased and was almost as high as the number of mast cells seen in systemic mastocytosis (20). Several studies have suggested that this increase in mast cell numbers in the gut may have clinical significance. For example, an increased number

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of mast cells in close proximity to mucosal nerves (within 5 μm of nerve fibers) was found to correlate with abdominal pain symptoms in patients with IBS (3). In addition, a recent study demonstrated that mast cell number and degranulation were significantly increased in patients with diarrhea-predominant IBS (36).

These studies and others support the contention that mast cells and their interactions with nerves play a role in the GI pathophysiology, but the mechanisms involved remain to be elucidated. There is substantial evidence that the release of neuropeptides from nerve ends is at least one pathway for nerve communication with mast cells. This is suggested by the finding that mast cells are frequently found closely apposed to small-caliber unmyelinated fibers (32) containing substance P and/or other neuropeptides (44), and many studies have shown that a variety of neuropeptides, including substance P, can elicit mast cell degranulation (10, 43). Although substance P is a potent mast cell-degranulating agent, it is not clear whether the action of substance P on mast cells is mediated through the high-affinity substance P receptor [neurokinin-1 (NK1)]. There is conflicting evidence in the literature regarding the mechanism of mast cell activation by substance P (15, 55). However, recent studies have shown that, under in vitro conditions, mast cells express NK1 receptors (31, 55). In addition, scant is the literature regarding the role of NK1 receptors in the nerve mast cell-relationship.

The goal of our studies was to determine whether nerve-mast cell relationships in the stomach and urinary bladder were influenced by the expression of NK1 receptors. Interestingly, we found a significant increase in the number of mast cells in tissues isolated from NK1 receptor knockout (NK1−/−) mice. Reconstitution of Kit mice with bone marrow cells isolated from NK1−/− mice resulted in the development of greater numbers of mast cells in tissues than in tissues of Kit mice reconstituted with bone marrow cells from normal donors. However, the spatial relationship between mast cells and nerves was maintained in the bladder in the absence of NK1 receptor expression. Together, these results indicate a regulatory effect of NK1 receptor expression on mast cell numbers in tissues, but no effect on the spatial relationship between mast cells and nerves in the bladder was shown.

**METHODS**

**Animals.** All animal experimentation described here was performed in accordance with the **Guiding Principles for Research Involving Animals and Human Beings** [Oklahoma University Health Sciences Center (OUHSC) Animal Care and Use Committee protocol 00-109] and the **Guiding Principles in the Care and Use of Animals of the American Physiological Society**. NK1−/− mice were generated by Dr. Norma Gerard and backcrossed to C57BL/6J mice. The colony at OUHSC was genotyped as described previously (12). Genetically mast-cell-deficient WBB6F1-Kit+/−/Kit+/− mice were purchased from Jackson Laboratories (Bar Harbor, ME). Both the wild-type mice for NK1 receptor mutation and congenic +/+ mice belong to the C57BL/6J background. Therefore, C57BL/6J mice were used as a control (+/+ for the NK1 and Kit mutations. Bone marrow- and mast-cell-reconstituted Kit mice (BMR Kit and MCR Kit mice, respectively) were generated as previously described (58, 59) (see below). Animals were maintained in animal housing facilities with filtered hooded cages and allowed food and water ad libitum.

**Bone marrow reconstitution of mast cell-deficient Kit mice.** Mice with double mutations at the W loci have a variety of phenotypic abnormalities, including a profound deficiency in the numbers of tissue mast cells, macrocytic anemia, age-dependent changes in intraepithelial lymphocyte populations in the GI tract, and other nonmegaloblastic abnormalities (57). Bone marrow transplantation repairs both the mast cell deficiency and the anemia of Kit mice (57). We utilized this approach to reconstitute Kit mice with +/+ and NK1−/− bone marrow cells. Briefly, femoral and tibial bone marrow cells from +/+ or NK1−/− mice were harvested in DMEM. The cells were washed three times and resuspended in DMEM, and 2 × 10⁵ bone marrow cells/mouse were injected intravenously into the mast cell-deficient Kit mice. Ten weeks later, the hematocrit was determined (to confirm that the mice had their anemia repaired), and these mice were then used for experiments.

**Mast cell reconstitution of mast cell-deficient Kit mice.** Briefly, femoral bone marrow cells from +/+ mice were maintained in culture for 4 wk in the presence of WEHI 3-conditioned medium (20% by volume) until mast cells represented >99% of the total cells as determined by neutral red staining. Two million mast cells in 0.2 ml of DMEM, or 0.2 ml of medium alone, were injected intravenously into Kit mice. Ten weeks later, the hematocrit was measured in all mice (to confirm that the mice were still anemic), and the mice were then used in experiments.

**Six groups of 10- to 12-wk-old female mice were used in experiments: C57BL/6J mice (+/+) used as control for Kit and NK1−/− mice, and mast-cell-deficient Kit mice, NK1−/− mice and Kit mice that were reconstituted with +/+ mast cells (MCR Kit), +/+ bone marrow cells (BMR Kit), and NK1−/− bone marrow cells (NK1−/− BMR Kit). A minimum of six mice were used per group.**

**Quantification of mast cell-nerve interaction.** Mouse bladders were immediately placed in 10% neutral buffered formalin for at least 48 h and then transferred into PBS (pH 7.8). Tissues were dehydrated in graded alcohol and xylene, and all of the bladders of the same group (n = 6) were then embedded together in paraffin as a multitissue block, according to conventional methods. Serial 5-μm sections were cut (8 μm apart), mounted onto SuperFrost Plus (Fisher Scientific, Pittsburgh, PA) microscopic slides, and dried overnight. Mast cells were stained according to Luna (27), and two serial sections of all blocks were quantified.

**Double histochemistry/immunohistochemistry.** The tissues were routinely processed for mast cell staining. Thereafter, the slides were subsequently processed for routine single immunohistochemistry (14). Slides were placed in PBS (pH 7.4) and treated with 3.0% H2O2 for 10 min at room temperature. All incubations (30 min) and washes were performed at room temperature. Normal blocking serum (Vector Labs, Burlingame, CA) was placed on all slides for 10 min. After a brief rinse in PBS, sections were treated with the neuronal-specific enolase (NSE) polyclonal antibody (Dako, Carpentaria, CA) at 1:2,000 dilution. After a wash in PBS, the avidin-biotin-horseradish peroxidase complex reagent (Vector Labs) was added. Slides were washed and were treated with 3,3′-diaminobenzidine (Biomedia, Foster City, CA) two times for 5 min, rinsed in distilled water, and counterstained with hematoxylin. Negative controls included replacement of the primary antibody with the antibody diluent (Zymed Laboratories, South San Francisco, CA) or use of the nonimmune serum (Vector Labs). We scanned histology slides using a Nikon digital camera (DXM1200, Nikon, Tokyo, Japan) mounted on a Nikon microscope (Eclipse E600, Nikon). Exposure times were held constant when images were acquired from different tissues. Images were imported into Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA) and then placed on Adobe Page Maker 7.0, in which groups of images were assembled and labeled. Composite figures were printed with Adobe Acrobat 6.01.

We performed morphometric analysis using the Neurolucida work-station for three-dimensional serial section reconstruction, morphometry, and image analysis (MicroBrightField, Williston, VT). Mast
cells presented a dark blue granular stain (light blue stomach epithelial cells were considered nonspecific artifactual staining), whereas the immunolabeled NSE-positive nerve fibers were brown. Mast cells were counted in all of the tissues with attention to the histological localization, such as mucosa, submucosal, and muscle.

The proximity of mast cells to NSE-positive fibers was scored as follows: close (mast cells closely apposed to nerve elements or within <50 μm of NSE-positive fibers) or far (>50 μm apart from NSE-positive fibers). It should be noted that all data were analyzed on two-dimension histological sections; therefore, we cannot rule out the possibility that mast cells considered close or far from NSE-positive fibers could be in contact with NSE-positive fibers via serial sectioning. Hence, because all tissues were analyzed identically, we made relative differences among the tissues.

Statistics: We conducted statistical analyses of histological data and immunohistochemistry using Wilcoxon’s rank sum test. Results are expressed as means ± SE (7), calculated using Prism 4.0 (GraphPad Software, San Diego, CA). The n values reported refer to the number of animals used for each experiment. In all cases, a value of P < 0.05 was considered indicative of a statistically significant difference.

RESULTS

The urinary bladder and glandular stomach of +/+ mice contained mast cells in the submucosa (Fig. 1, A and B), smooth muscle (Fig. 1, C and D), and the adventitia (Fig. 1E). Mast cells were found in proximity to immunolabeled, NSE-positive nerve elements in both the urinary bladder (Fig. 1, A–E) and the glandular stomach (Fig. 1, F–I). However, as defined with light microscopy, the pattern of contact between the mast cell and the nerve element varied. There were sites where a single mast cell appeared to be in direct contact with a single nerve element (Fig. 1A). In contrast, there were sites where individual mast cells appeared in contact with several NSE-positive segments (Fig. 1D), whereas at other sites multiple mast cells appeared to contact a single NSE-positive segment (Fig. 1, E, H, and I). Mast cells also appeared to be in direct contact with smooth muscle cells (Fig. 1H), around blood vessels (Fig. 1A), and with other mast cells (Fig. 1G). As reported in rats (48), mast cells in the glandular stomach of mice appeared to be in close proximity to nerve elements in the subepithelial layer (Fig. 1F), the submucosal plexus (Fig. 1G), the myenteric plexus (Fig. 1H), and the adventitia (Fig. 1I). We also confirmed the mast cell deficiency of Kit mice: no mast cells were identifiable in either the bladder or the glandular stomach.

To assess the influence of NK1 receptor expression on mast cell numbers and their contact with nerve elements, we determined the density of mast cells in the urinary bladder and the glandular stomach in NK1−/− mice. At the light microscopic level, we found an increase in the total number of mast cells at these sites (Figs. 2 and 3). Representative photomicrographs of the distribution of mast cells in the urinary bladder and glandular stomach of NK1−/− mice are presented (Figs. 2, A–C, and 3A, respectively). Quantification of mast cells in the bladder of NK1−/− mice demonstrated a sixfold increase compared with +/+ mice (140 ± 14 vs. 9.6 ± 1 mast cells/mm², respectively, P < 0.05; Fig. 4). Similarly, the glandular stomach of NK1−/− mice exhibited an increased number of mast cells compared with +/+ mice (50 ± 5 vs. 19 ± 1 mast cells/mm², respectively, P < 0.05; Fig. 4).

We next determined the effect of the absence of mast cell expression of NK1 receptors on tissue mast cell numbers by systemically transplanting either bone marrow cells or bone marrow-derived mast cells from NK1−/− mice into mast cell-deficient Kit mice. We found that either approach successfully replenished mast cells in both the bladder and the glandular stomach of Kit mice. Representative photomicrographs of the distribution of reconstituted mast cells are shown in the urinary bladder (Fig. 2G) and in the glandular stomach (Fig. 3E and F).

The relative effectiveness of each method with regard to the reconstitution of mast cells in Kit mice was assessed. That is, would bone marrow cells from NK1−/− mice have the same capacity to reconstitute Kit mice as +/+ bone marrow cells or +/+ in vitro-derived mast cells? When we quantified the number of tissue mast cells using these different donor cell populations, it was found that transplantation of either NK1−/− bone marrow cells, +/+ bone marrow cells, or +/+ in vitro-derived mast cells into Kit mice led to marked increases in the number of bladder mast cells (as great as 3 times the number of mast cells seen in the bladder of +/+ mice) (Fig. 4). A marked increase in mast cell numbers was also found in the glandular stomach of Kit mice after the administration of NK1−/− bone marrow cells or +/+ in vitro-derived mast cells but not +/+ bone marrow cells (Fig. 4).

We next examined the distribution of mast cells in the mucosa and muscle layers of the urinary bladder and glandular stomach. In +/+ mice, mast cells were more numerous in the muscle layer compared with the mucosa of the bladder (Fig. 5A). By contrast, mast cells in the glandular stomach were more numerous in the mucosal layer than in the muscle (Fig. 6A). The percentage of total mast cells in the mucosa and muscle layer of the bladder of NK1−/− mice remained constant (Fig. 5A), whereas there was a significant increase in the percentage of mast cells found in the mucosa of the glandular stomach of NK1−/− mice (Fig. 6A). Reconstitution of Kit mice with bone marrow cells from +/+ or NK1−/− mice resulted in a greater percentage of total mast cells in the bladder taking residence in the mucosa of the bladder (Fig. 5A), whereas the percentage of mast cells in the reconstituted glandular stomachs was similar to that seen in +/+ mice (Fig. 6A). Reconstitution of Kit mice with +/+ in vitro-derived mast cells led to a distribution of cells in both the bladder and the glandular stomach, which was similar to that found in +/+ mice (Figs. 5A and 6A).

We then assessed the spatial relationship between mast cells and nerves in each of the above groups. With regard to the urinary bladder, although relatively few mast cells were found in the mucosa of +/+ mice (an average of 3.6 mast cells/mm² of cross-sectional area), virtually 100% of them appeared closely apposed to a nerve element (Fig. 5B). A similar distribution was observed in the detrusor layer of the bladder in +/+ mice (Fig. 5C). By contrast, the majority of the mast cells in the mucosa and muscle of the bladder of NK1−/− mice was closely apposed to a nerve element (Fig. 5, B and C). However, in both sites, NK1 deletion resulted in an increase in the number of mast cells that were far (>50 μm) from nerve elements.

In the glandular stomach of +/+ mice, although there was a strong association between mast cells and nerves, it was not as frequent as seen in the bladder. Approximately 70% of the mast cells in the mucosa of the stomach and 58% in the muscle layer appeared closely apposed to a nerve element (Fig. 6, B
and C). In addition, the deletion of the NK1 receptor did not appear to have the same effect on mast cell-nerve relationships in the stomach as it had in the bladder. Although there appeared to be a diminution in the percentage of mast cells in direct contact with nerve elements (B-D), mast cells with blood vessels (A and E), and mast cell-mast cell contact (E and G). Blue-stained mast cells were histochemically stained with toluidine blue, whereas brown-labeled nerve elements were immunohistochemically detected with neuronal-specific enolase (NSE) polyclonal antibody using the HRP-ABC-DAB detection system. White arrowheads indicate nerve elements, black arrowheads indicate mast cells, and black arrows indicate blood vessels. All micrographs were taken at ×400.

Finally, we analyzed the spatial relationship between mast cells and nerves in the different models of mast cell reconstitution of Kit mice. The systemic reconstitution of Kit mice with +/+ bone marrow cells resulted in similar mast cell-nerve spatial relationships as seen in +/+ mice in the mucosa and muscle of the bladder (Fig. 5, B and C) and stomach (Fig. 6, B and C). Only in the glandular stomach of these Kit reconstituted mice did it appear that there was more contact between mast cells and nerves (Fig. 6B).

Examination of the bladder of Kit mice reconstituted with bone marrow from NK1−/− mice revealed a lower percentage of mast cells in contact or in proximity to nerves in the bladder mucosa (Fig. 5B), whereas a greater percentage appeared in direct contact in the muscle layer (Fig. 5C). Interestingly, this
reconstitution procedure reestablished very similar mast cell-nerve spatial arrangements in the mucosa and muscle of the glandular stomach (Fig. 6, B and C).

Finally, there was no evidence that culturing bone marrow cells to derive immature mast cells for reconstitution of Kit mice (MCR Kit) changed their capacity to take up anatomic relationships with nerve elements. In the bladder, MCR Kit mice had similar percentages of mast cells closely apposed to nerve elements compared with BMR Kit mice in both the mucosa and muscle layers (Fig. 5, B and C). The same was found in the mucosa (Fig. 6B). However, in the stomach muscle, it seemed that in vitro-derived mast cells ultimately developed closer proximity to nerve elements than did mast cells that arose from the bone transplantation (Fig. 6C).

DISCUSSION

Mast cells are a normal resident of almost all vascularized tissues in mammalian species and play a crucial role in inflammatory conditions involving the bladder (39–41) and GI tract.
In these tissues and other sites, mast cells have close anatomic proximity to lymphatics (56), vascular endothelial cells (21), and nerve elements (22), but the factors that potentially influence mast cell interactions with other cells are not completely understood.

We chose to examine mast cell-nerve relationships using mouse bladder and stomach as model tissues. The rationale for examining and comparing mast cells at these sites was based on evidence suggesting that mast cells may modulate a cross-sensitization between GI and bladder inflammation (38). For example, patients having IBS also often suffer from urological dysfunction, and IBS occurs quite frequently in patients with interstitial cystitis (1). We also chose to examine mast cells in the mucosal and muscular layers of the bladder and glandular stomach, primarily because the mast cells at these sites are phenotypically distinct. This property of phenotypic heterogeneity of mast cells at mucosal sites (mucosal-type mast cells) compared with mast cells in the muscularis propria (connective tissue-type mast cells) has been long appreciated, particularly in rodent species, and may have important functional implications (16, 42, 43).
The present study identified a number of novel observations concerning mast cell-nerve interactions in the noninflamed tissues of the mouse urinary bladder and the glandular stomach. We report a marked expansion of mast cell numbers in the bladder and glandular stomach of mice genetically deficient in the expression of the high-affinity receptor for substance P (NK1). However, these mice maintained the spatial arrangement between mast cells and nerves, indicating that this receptor does not play an essential role in the modulation of nerve-mast cell interactions under normal conditions. It remains to be determined whether NK1 receptors play a different role in the inflamed tissue.

In addition, we demonstrated that transplanting mast cells or mast cell precursors into mast cell-deficient Kit mice results in the repopulation of mast cells in the urinary bladder and glandular stomach and that these mast cells take up the anticipated spatial relationships with nerve elements. The proliferation, survival, recruitment, and maturation of mast cell precursors are influenced by several cytokines and growth factors, including, but not limited to, IL-3, IL-4, IL-9, stem cell factor, nerve growth factor, transforming growth factor-β, and some nongrowth factors such as IgE antibodies (2, 24, 46). Most of these factors have been studied in vitro or in the context of inflammation. However, the precise factors maintaining mast cell number under normal physiological conditions are not completely defined. It is clear that stem cell factor and its receptor c-kit are essential for the development of normal numbers of tissue mast cells, as demonstrated by the almost complete lack of mast cells in stem cell factor-deficient (Mgflt/LtMgflt/Mgflt-d) and c-kit-deficient (Kitl+/Kitl+/+) mice (18, 23, 57). The findings of increased numbers of tissue mast cells in normal bladder and stomach of NK1−/− mice suggest a negative regulatory role of NK1 receptor expression, but the precise mechanism remains to be determined. Mast cells are thought to have extended half-lives in tissues (34), but the expansion of mast cell populations can be achieved by several possible processes, including recruitment of precursor cells, the local proliferation of the resident mast cells, and the migration of tissue mast cells (54). Additional studies will be needed to

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determine which of these mechanisms are responsible for the increased number of tissue mast cells seen in NK1−/− mice.

In addition, the percentage of mast cells that were present in the bladder mucosa and muscle layer of NK1−/− mice remained similar to that seen in +/+ mice, although there was a significant increase in the percentage of mast cells occupying the mucosa of the glandular stomach. The reason for this relative increase in gastric mucosal mast cells is not clear. In vitro studies have indicated that a balance of factors, such as IL-3 and IL-10, are important in driving mast cell differentiation (2). We speculate that this balance of cytokines may be skewed and/or enriched in the gastric mucosa, thus promoting a greater expansion of mast cell populations at this site.

Multiple approaches for the reconstitution of mast cells in mast cell-deficient Kit mice were used in our study, and most resulted in an increase in the number of mast cells found in the bladder and glandular stomach. These findings suggest that these tissues have the ability to accommodate increased numbers of mast cells if additional precursor cells or immature mast cells are provided. This is not surprising because the number of mast cells in tissues is frequently increased in various chronic inflammatory conditions (16, 19, 20, 25, 51–53). However, our findings would indicate that tissue mast cell numbers are expandable even under normal physiological conditions.

In addition to the change in the number of mast cells, we also assessed the spatial relationship between mast cells and nerves at a light microscopic level. Although many mast cells appeared to be in direct contact with nerve elements, it was technically difficult to precisely determine whether direct cell-cell membrane contact actually occurred at this degree of magnification. We therefore considered two categories, as defined by the distance between the mast cell and nerve. The first consisted of cells that appeared to be in direct contact or in close proximity to one another (<50 μm; termed close) and the second category consisted of cells that had a distance between them of >50 μm (termed far). This type of categorization has been used by other investigators (8, 48) and may be indicative of functionally meaningful interactions between mast cells and nerves.

With this in mind, there were several findings in our study. In the absence of NK1 receptor expression, the spatial relationship between mast cells and nerves was essentially maintained. In the glandular stomach of NK1−/− mice, ~70% of mucosal mast cells and 60% of mast cells in the muscularis propria exhibited apparent direct contact or close proximity to nerve elements (compared with 70% and 58%, respectively, in +/+ mice). There were some differences regarding the urinary bladder of NK1−/− mice, with ~80% of mucosal mast cells and 65% of mast cells in the detrusor muscle spatially related to nerve elements (compared with 100% in +/+ mice). However, it is not clear whether this slight decrease in mast cell-nerve relationship represents normal biological variability with no functional significance. The repopulation of mast cells in Kit mice with bone marrow derived from NK1−/− mice resulted in similar mast cell-nerve spatial relationship in the mucosa and muscle layers of the stomach and the muscle layer of the bladder. This spatial arrangement was diminished to 50% of that seen in +/+ mice. This finding suggests that mast cells in the mucosa of the bladder may be more dependent on the expression of an NK1 receptor for the development of anatomic relationship with nerves. In all other transplantation approaches utilized, although there were minor differences between procedures and the anatomic sites, the spatial relationship between mast cells and nerves remained relatively consistent with that seen in normal mice.
Our results indicate that NK$_1$ receptor expression affects the number of mast cells seen in the glandular stomach and urinary bladder of mice. However, the NK$_1$ receptor appears to have little influence on the spatial relationship between mast cells and nerves. Maintenance of this anatomic relationship may be critical for a broad range of mast cell inflammatory and immunoregulatory activities.

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REFERENCES


28. Malaviya R, Ikeda T, Ross E, and Abraham SN. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TGF-$


