RANTES mediates TNF-dependent lamina propria mast cell accumulation and barrier dysfunction in neurogenic cystitis

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Chen MC, Keshavan P, Gregory GD, Klumpp DJ. RANTES mediates TNF-dependent lamina propria mast cell accumulation and barrier dysfunction in neurogenic cystitis. Am J Physiol Renal Physiol 292: F1372–F1379, 2007. First published January 23, 2007; doi:10.1152/ajprenal.00472.2006.—Barrier dysfunction of the urinary bladder is postulated to contribute to patient morbidity in the bladder inflammatory disease interstitial cystitis (IC). IC is often considered a neurogenic cystitis, but the mechanisms underlying barrier dysfunction are unclear. In murine neurogenic cystitis induced by pseudorabies virus (PRV), we previously observed formation of urothelial lesions characterized by urothelial apoptosis and urothelial discontinuities. Lesion formation was preceded by mast cell trafficking to the lamina propria, and trafficking was mediated by tumor necrosis factor-α (TNF). Here, we found that supernatants of TNF-treated wild-type mice induced RANTES expression in the urothelium that was temporally coincident with lamina propria mast cell accumulation (maximum at days 3–4 following infection) and was not induced in TNF−/− mice, TNFR1/2−/− mice, or mice treated with anti-TNF antibodies. Anti-RANTES antibodies blocked PRV-induced lamina propria mast cell accumulation 56% and reduced the prevalence of animals with detectable lesions 42%, relative to isotype control antibodies. Bladder barrier function was quantified by measuring transepithelial resistance; psoriasis; irritable bowel disease are increasingly understood and have given rise to novel and effective treatment options that restore epithelial integrity in IC patients with mast cell-associated disease.

Transplantation resistance  

**EPITHELIAL INTEGRITY IS ESSENTIAL FOR ORGAN FUNCTION** and protecting the host from environmental factors. Loss of epithelial integrity in diseases such as psoriasis, Crohn’s disease, and possibly bladder interstitial cystitis (IC) causes major patient morbidity and is a significant burden on the healthcare system (21, 38, 43). The mechanisms underlying psoriasis and irritable bowel disease are increasingly understood and have given rise to novel and effective treatment options that restore epithelial integrity for these patients. Although the bladder also exhibits epithelial lesions in many IC patients, the mechanism by which barrier dysfunction occurs in IC in the absence of infection is largely unknown.

IC is a chronic, debilitating disease of the bladder that afflicts ~1 million patients in the United States and is characterized by urinary frequency and urgency, nocturia, and bladder/pelvic pain (23, 26). Evidence for a loss of urothelial integrity in IC includes ulcerations and glomerulations (pin-point hemorrhages) visible during cystoscopy, urothelial lesions and discontinuities visible in biopsies, and increased sensitivity to instillation of KCl (22, 29, 37, 46). IC is often considered a neurogenic cystitis because patients exhibit aberrant voiding activity, and neuromodulatory therapies show partial efficacy, suggesting a neural component. Mast cells are also thought to play a role in the pathogenesis of IC for at least a subset of patients because mast cell counts are elevated in the bladders of some IC patients, and histamine metabolites and mast cell tryptase are increased in some patient urines (7, 18). Indeed, the IC Database Study (ICDB) identified correlations between urothelial lesions and mast cell accumulation in the lamina propria and the IC symptoms of nocturia, urgency, and 24-h frequency (29, 46). Although ICDB participants were individuals with classic, advanced disease that may represent only a subset of IC patients, the mechanism by which mast cells accumulate in the lamina propria and contribute to loss of urothelial integrity remains unclear.

Several factors that mediate mast cell chemotaxis have been identified, including eotaxins, stromal-derived factor, stem cell factor, and CCL5/RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) (50). RANTES is a well-characterized mast cell chemokine and has been shown to drive mast cell trafficking to the gut and lung (41). RANTES mediates its chemotactic effects by interacting with G protein-coupled receptors CCR1/3/4/5 expressed on the mast cell surface (27, 34, 35), and CCR3 is required for appropriate trafficking of mast cells to the gut and lung (16). Although CCR1 and CCR5 expression on kidney macrophages has been reported (20), the chemokine(s) that drive mast cell trafficking in the lower urinary tract are currently unknown.

To elucidate the mechanisms of neurogenic cystitis, a murine model was developed based on infection with the attenuated Bartha’s strain of the neurotrophic α-herpes virus pseudorabies virus (PRV). Previous studies in rat demonstrated that Bartha’s PRV infection of the abductor caudalis (tail base) muscle resulted in cystitis associated with mast cell influx, even though the Bartha’s strain failed to reach the bladder due to defects in anterograde viral transport (17, 24, 45). Using the murine model, we observed that neurogenic cystitis induces mast cell-induced formation of urothelial lesions with a comitant decrease in transepithelial resistance (TER), and both processes are dependent on TNF (12). We previously demonstrated that murine neurogenic cystitis results in vascular per-
meability and non-PMN leukocyte infiltration (11) in the absence of gross histological changes in the bladder, such as the hemorrhagic cystitis observed in the rat model (25). Accumulation of mast cells in the lamina propria was also observed, reminiscent of the lamina propria mast cells accumulation that is associated with symptoms in human IC, and this accumulation was dependent on TNF in the murine model. Based on these observations, we hypothesize that TNF induces the expression of a mast cell chemokine during neurogenic cystitis, which then mediates mast cell trafficking to the lamina propria. Identifying such a chemokine would provide insights to the mechanisms underlying the loss of urothelial integrity in neurogenic cystitis.

In this study, we identify RANTES/CCL5 as a TNF-induced urothelial chemokine that promotes mast cell chemotaxis. We demonstrate that antibody therapy against RANTES effectively blocks the accumulation of lamina propria mast cells and stabilizes bladder barrier function. Therefore, these data suggest a mechanism for barrier dysfunction in neurogenic cystitis and identify potential strategies for the diagnosis and treatment of IC in patients with mast cell-associated disease.

**METHODS**

**Animals.** Female 4- to 6-wk-old C57BL/6J mice or mice with targeted deletion of TNFR1 and TNFR2 (TNFR1/2−/−; B6.1295-Tnfrsf1a−/−; Tnfrsf1b−/−) were purchased from Jackson Laboratory (Bar Harbor, ME). TNF-deficient mice (TNF−/−) were generously provided by Dr. M. Brown (Northwestern University, Chicago, IL). TNF−/− mice were backcrossed 10 generations to C57BL/6J mice. All experiments were performed using protocols approved by Northwestern University Animal Care and Use Committee. The mice were housed in containment facilities of the Center for Comparative Medicine and maintained on a regular 12:12-h light-dark cycle with food and water ad libitum.

**Cell culture.** PD07i and SR22A cell lines were generated by immortalization of primary cultures, established from normal and IC urothelium, respectively, with LSNX HPV16 E6E7, as previously described (12, 30). Cell lines were maintained in serum-free EpiLife medium (Cascade Biologics, Portland, OR). In experiments using TNF, recombinant human TNF-α (cat. no. 654205; EMD Biosciences, La Jolla, CA) was added to EpiLife at a final concentration of 1 ng/ml.

Mouse bone marrow mast cells were generated by culturing primary bone marrow cells from female WBB6/F1/J mice. Bone marrow was dissociated by trituration. The cell suspension was plated onto plastic culture dishes and grown in complete RPMI media (15% heat-inactivated fetal bovine serum, 2 mM glutamine, 1 mM pyruvate, 50 μM β-mercaptoethanol) containing 5 ng/ml recombinant IL-3 (Biosource, Camarillo, CA). Throughout the first 8 wk of culture, 12 ng/ml recombinant murine SCF (Biosource) was added to the culture, and differentiation into a mature mast cell phenotype was confirmed by c-kit (CD117) and FceRI (IgE receptor) expression as well as toluidine blue staining.

**Induction of neurogenic cystitis.** Bartha’s strain of PRV was propagated on porcine PK15 cells and titrated using standard techniques (9). Neurogenic cystitis was induced in mice by injection of 10 μl of PRV containing 2.29 × 106 pfu into the abductor caudalis dorsalis (tail-base) muscle with a 26-gauge Hamilton syringe while maintaining the animals under anesthesia as previously described (11). Ultraviolet-irradiated PRV stocks were employed as negative control inocula in sham-treated animal groups. For experiments employing antibody treatments, mice were administered 250 μg anti-mouse/rat TNF monoclonal antibody (TN3–19.12; BD Pharmingen, San Jose, CA) or 30 μg anti-rat CCL5/RANTES monoclonal antibody (clone 53405.111; R&D Systems, Minneapolis, MN) via intraperitoneal injection 1 day before and 1 day after PRV infection. Purified hamster IgG1 monoclonal antibody (G235–2356; BD Pharmingen) or rat IgG2A (R35–95, BD Pharmingen) was used as isotype control for anti-TNF and anti-RANTES therapy experiments, respectively. Animals were monitored for full recovery from anesthesia and were euthanized at various times for tissue harvest.

**Antibody array identification of putative mast cell chemokines.** Antibody arrays (RayBio Human Cytokine Antibody Arrays VI and VII, RayBiotech) were performed according to the manufacturer’s protocol using culture supernatants from unstimulated SR22A cells and cells stimulated with TNF for 4 h. The array films were scanned in transparency mode using a Perfection 2450 flatbed scanner (Epson), and spot intensities were determined using NIH Image software. All spot intensities were initially normalized to internal control spots on each filter, and then ratios of spot intensities for induced cultures relative to uninduced cultures (+TNF at 4 h/−TNF) were calculated. Factors with intensity ratios greater than 2.0 were chosen for further study.

**Mast cell chemotaxis assay.** Chemotaxis was assessed using an APTM Micro Chemotaxis Chamber in conjunction with a polycarbonate filter (8-μm pores; Neuro Probe, Gaithersburg, MD). Briefly, cell culture supernatants were placed into the lower chambers. Filters were coated with 1 μg/ml fibronectin in PBS for 30 min at room temperature and subsequently placed over the lower chamber, and the chamber was assembled. Upper chambers were filled with 5 × 104 BMMCs/well in 50 μl complete RPMI media. The apparatus was incubated at 37°C for 4 h. Nonmigrating cells were removed by gentle scraping of the upper filter surface. Filter was fixed in 95% ethanol, stained with DAPI, and mounted onto a microscope slide. Cells that migrated into the filter were detected by fluorescence microscopy, and nuclei were counted in five ordered fields (×20 magnification). Field counts were averaged to represent a mean migration value for each well. Triplicate wells will be counted for each experimental condition, and reported values represented the experimental values less the value of chemokinesis control wells. In some experiments, culture supernatants were pretreated at room temperature with neutralizing antibodies against TNF (1 μg/ml), RANTES (1 μg/ml), or Eotaxin-1 (10 μg/ml) before the chemotaxis assays.

**Immunohistochemical analysis.** Bladder tissue sections were deparaffinized and rehydrated. Subsequently, RANTES immunoreactivity was detected using 1:10 purified rat monoclonal anti-CCL5/RANTES antibody (clone 53405; R&D Systems) in combination with Dako-Cytomation ARK kit (Dako, Carpenteria, CA). Staining procedures were performed according to manufacturer’s protocol. Images were acquired using a Nikon E800 microscope equipped with a Spot Color RT camera.

**TUNEL staining.** Urothelial apoptosis was assessed in tissue sections with TUNEL as previously described (12). Briefly, sections were deparaffinized, rehydrated, and incubated with 20 μg/ml nuclelease-free proteinase K at 37°C. Sections were then rinsed with PBS and incubated at room temperature with blocking solution (3% BSA, 20% FBS). Following incubation with TUNEL reaction mixture (Roche Diagnostics, Indianapolis, IN), slides were counterstained with DAPI, and images were acquired using a Nikon E800 microscope equipped with a Spot Color RT camera (Diagnostic Instruments, Sterling Heights, MI). A putative lesion was defined as three or more adjacent TUNEL-positive cells. To confirm lesions in tissue sections with 3+ contiguous, TUNEL-positive cells, coverslips were removed, and sections were stained for uroplatin III (12). Regions exhibiting 3+ TUNEL-positive cells and associated with a disruption in the normally continuous staining for uroplatin III (i.e., uroplatin III discontinuity at the site of apoptosis) were scored as lesions. Lesions were scored by staining of two nonserial sections from each bladder.

**Acidified toluidine blue staining.** Tissue sections were deparaffinized, rehydrated, and stained with acidified toluidine blue to visualize mast cells, as described previously (12). Briefly, tissue sections were immersed in 0.5% potassium permanganate. After being rinsed


in distilled water, sections were immersed in 2% potassium metabisul
fite, incubated in tap water, and washed with distilled water. Last, sections were immersed in 0.02% toluidine blue in 0.25% glacial acetic acid, pH 3.2. To quantify lamina propria mast cells, toluidine blue-stained cells were counted in the lamina propria of two nonadjacent tissue sections and reported as the mean of sections with sections that were scored positive for lesions or RANTES staining and assigning a value of 0 to bladders with sections absent in lesions/RANTES. Statistical significance was calculated using one-
way ANOVA followed by a posttest comparison using Bonferroni multiple comparison test. All values of \( P < 0.05 \) were considered statistically significant.

RESULTS

Urothelial RANTES secretion mediates mast cell chemotaxis in vitro. We hypothesized that TNF induces the expression of a mast chemokine within the bladder that directs accumulation of mast cells near the urothelium. To test this possibility, we developed a mast cell chemotaxis assay using primary, murine bone marrow-derived mast cells (BMMCs) in conjunction with a modified Boyden chamber. To initially determine which cell type best directed BMMC chemotaxis in vitro, pilot experiments were conducted with cell cultures representing each of the bladder tissue layers [TEU-1, PDO7, and SR22A urothelial cells (12, 28); 3T3 and bladder BSM-2 cells (32) to mimic lamina propria fibroblasts; and primary detrusor smooth muscle cells (5)]. Culture supernatants of the immortalized human urothelial cell line SR22A, derived from the bladder of an IC patient (30), induced the greatest chemotaxis (data not shown). Culture supernatants of TNF-stimulated SR22A cells induced time-dependent BMMC migration that was maximal at 4 h (Fig. 1A; \( P < 0.001 \)) and exceeded the chemotactic effect of TNF alone (\( P < 0.05 \)). To provide a preliminary identification of TNF-induced urothelial chemokines that potentially mediate BMMC chemotaxis, chemokine antibody arrays were probed with SR22A culture supernatants from unstimulated cultures and cultures treated with TNF for 4 h. Eotaxin-1, RANTES/CCL5, and TNF were identified as putative mast cell chemokines that were elevated more than twofold in supernatants of urothelial cultures treated with TNF relative to control supernatants (Table 1). To confirm a role for RANTES in mast cell chemotaxis, chemotaxis assays were performed using TNF-

![Fig. 1. TNF induces murine bone marrow-derived mast cell (BMMC) chemokines. A: \( 5 \times 10^4 \) BMMCs were placed into the top well of a modified Boyden chamber with an 8-µm-pore filter. The bottom well contained serum-free media alone (–), serum-free media with 1 ng/ml TNF (TNF), and conditioned media of SR22A cultures stimulated with TNF for 30’, 60’, or 4 h. Chemotaxis was assessed by counting 5 high-power fields within an individual well, and data for each experimental condition are presented as means ± SE of triplicate wells. The chemotactic effect of SR22A culture supernatants was time dependent following addition of TNF (*\( P < 0.001 \)). B: chemotaxis assay was repeated using SR22A cultured supernatants that were pretreated with either isotype antibody (control) or neutralizing antibodies against Eotaxin-1, TNF, and RANTES. Culture supernatants from TNF-stimulated SR22A cells treated with neutralizing antibodies against RANTES or Eotaxin-1 significantly reduced mast cell chemotaxis (**\( P < 0.001 \) and *\( P < 0.01 \), respectively). All results are representative of 3 independent experiments.](http://ajprenal.physiology.org/)

Table 1. Putative TNF-induced mast cell chemokines secreted by urothelial cultures

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<tr>
<th>Factor</th>
<th>Fold Induction*</th>
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<tr>
<td>Eotaxin-1</td>
<td>10.4</td>
</tr>
<tr>
<td>TNF</td>
<td>2.3</td>
</tr>
<tr>
<td>RANTES/CCL5</td>
<td>2.2</td>
</tr>
<tr>
<td>Stem cell factor</td>
<td>1.7</td>
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<tr>
<td>IL-6</td>
<td>1.3</td>
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Microarray spot intensity of 4-h TNF-stimulated supernatants normalized to intensity of 0-h TNF-stimulated spots.

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stimulated SR22A culture supernatants that were subsequently treated with neutralizing antibodies specific for RANTES (Fig. 1B). Whereas isotype control and anti-TNF antibodies had no effect, antibodies against RANTES and Eotaxin-1 significantly reduced BMMC chemotaxis in response to urothelial culture supernatants (P < 0.001 and P < 0.01, respectively).

Neurogenic cystitis induces urothelial RANTES in vivo. RANTES has previously been shown to act as a chemokine important for homing of mast cells to the lung and the gut (1, 41). To determine whether RANTES plays a role in neurogenic cystitis, we examined bladders of wild-type (B6) mice for RANTES expression at various times after PRV infection (41). To determine whether RANTES plays a functional role in the bladder, we examined the effect of anti-RANTES antibodies on lamina propria mast cell accumulation relative to control antibodies (40/5, P < 0.01) and TNF-1 mice (0/5, P < 0.01) did not exhibit RANTES immunoreactivity at PID 3 in either the urothelium or the detrusor (Fig. 3C). These data suggest that mast cell TNF secretion induces urothelial RANTES expression during neurogenic cystitis.

Anti-RANTES antibodies block mast cell trafficking and urothelial lesion formation. To determine whether RANTES plays a functional role in the bladder, we examined the effect of anti-RANTES antibodies on lamina propria mast cell accumulation during neurogenic cystitis. Mice were administered two doses of neutralizing anti-RANTES antibody (30 μg/dose), PID –1 and PID 1 relative to PRV infection. Control groups received either sham injections with saline or injections of isotype control antibody, and bladders from all mice were harvested for analysis at PID 3 (Fig. 4A). Consistent with our previous observations (11, 12), infection resulted in significant accumulation of lamina propria mast cells in PRV-infected mice relative to sham-treated mice (P < 0.01), and isotype control treatment had no effect on accumulation. Anti-RANTES antibodies, however, significantly inhibited lamina propria mast cell accumulation relative to control antibodies (P < 0.001).

Table 2. TNF signaling mediates RANTES expression in urothelium

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<tr>
<th>Group</th>
<th>RANTES Immunoreactivity</th>
<th>Significance</th>
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<tbody>
<tr>
<td>+/+</td>
<td>4/5</td>
<td></td>
</tr>
<tr>
<td>TNFR1/2−/−</td>
<td>0/5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>TNF−/−</td>
<td>0/5</td>
<td>P &lt; 0.01</td>
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Fig. 2. Neurogenic cystitis induces urothelial RANTES. Wild-type mice were infected with pseudorabies virus (PRV), bladders were harvested at postinfection day (PIDs) 0–5, and sections were stained for RANTES. The bladder was visually subdivided into 4 layers: urothelium (U), lamina propria (LP), proximal half of the detrusor muscle relative to the bladder lumen (D1), and the distal half of the detrusor relative to the bladder lumen (D2). A: mice harvested at PID 0 displayed no signs of RANTES immunoreactivity. B: faint RANTES immunostaining was present only in the detrusor in PID 1 animals. C: at PID 2, RANTES staining was detected in the urothelium (arrowhead). D and E: urothelial RANTES immunoreactivity was strongest at PID 3–4, respectively. Staining in the detrusor decreased between PID 3 to PID 4. F: PID 5 animals were largely absent of RANTES staining in the urothelium. Scale bar (A-F) represents 50 μm.
Although we cannot exclude the possibility that anti-RANTES antibodies acted directly on the urothelium, these data are consistent with a role for RANTES as a functional mediator of mast cell trafficking in neurogenic cystitis.

Since we previously reported leukocyte influx during murine neurogenic cystitis (10), we examined whether RANTES mediated accumulation of leukocytes (Fig. 4). Both PMN and non-PMN leukocytes were quantified in sham-infected wild-type mice and in PRV-infected mice treated with either anti-RANTES or isotype control antibodies. PRV infection resulted in significantly higher counts of non-PMN leukocytes relative to sham-infected mice ($P < 0.01$), and counts were not different between PRV-infected mice receiving control antibodies or anti-RANTES. These data suggest that general inflammatory responses during neurogenic cystitis were not altered by the administration of anti-RANTES antibody, supporting the possibility that anti-RANTES acts specifically at the level of mast cell trafficking.

Our previous studies indicated that TNF was required both for mast cell trafficking and for urothelial lesion formation via apoptosis (12). Since both processes are TNF dependent, it was not previously possible to dissect the contribution of mast cell trafficking to urothelial apoptosis. To assess the contribution of mast cell trafficking in urothelial lesion formation, bladder tissues from PRV-infected mice at PID 3, either treated with blocking anti-RANTES antibody or isotype control antibody, were stained for apoptotic nuclei by TUNEL. Urothelial integrity was also determined in the same tissue sections by staining for uroplakin III, a superficial urothelial cell marker (48, 49). In sham-treated mice, no TUNEL-positive nuclei were observed.
and uroplakin staining was continuous along the superficial urothelial cell layer, indicating intact urothelium and an absence of apoptosis (Fig. 5A). PRV-infected mice receiving control antibodies exhibited foci of apoptotic nuclei that were also associated with discontinuities of uroplakin staining that represented urothelial lesions (Fig. 5B). While occasional TUNEL-positive cells and uroplakin discontinuities were also observed in the urothelium of PRV-infected mice receiving anti-RANTES treatment, these lesions were typically less severe than animals receiving control antibodies (compare Fig. 5, B and C). These findings were supported by a significantly lower prevalence of lesion-positive animals in mice receiving anti-RANTES antibodies relative to controls (Table 3). Therefore, these data suggest that trafficking of mast cells to the lamina propria contributes to TNF-dependent urothelial apoptosis and the formation of urothelial lesions.

**Anti-RANTES antibodies stabilize bladder barrier function.** Previously, we demonstrated that PRV-induced neurogenic cystitis was associated with a loss of bladder barrier function, and anti-TNF antibodies stabilized barrier function (12). These observations confirmed the functional significance of TNF-dependent urothelial apoptosis and lesion formation. To determine whether RANTES-mediated mast cell trafficking also plays a role in the loss of bladder barrier function, TER was quantified in bladders of mice receiving anti-RANTES antibodies or isotype control antibodies (Fig. 6). PRV-induced neurogenic cystitis resulted in a significant decrease in TER at PID 5 relative to sham-infected mice (P < 0.01) that was not altered by treatment with isotype control antibodies. In contrast, PRV-infected mice treated with anti-RANTES antibody exhibited TER that was significantly higher than mice receiving control antibodies (P < 0.01). These functional data support our microscopic findings of reduced lesions in mice treated with anti-RANTES and suggest anti-RANTES therapy stabilizes bladder barrier function by blocking mast cell trafficking.

**DISCUSSION**

RANTES is central to the pathogenesis of inflammatory diseases of both the skin and the gut, including atopic dermatitis, psoriasis, and inflammatory bowel disease (3, 19, 42), but the role of RANTES in the pathogenesis of inflammatory bladder diseases is unknown. In this study, we identify a critical role for RANTES in neurogenic cystitis. Our results show that RANTES mediates TNF-dependent mast cell accumulation in the lamina propria, which contributes to urothelial lesion formation and bladder barrier dysfunction. Neutralizing antibodies specific for RANTES blocked lamina propria mast cell accumulation, abrogated urothelial lesion formation, and stabilized barrier dysfunction. These results indicate that RANTES is central to the pathogenesis of neurogenic cystitis and suggest that chemokines, such as RANTES, represent novel therapeutic targets for the treatment of bladder inflammation.

**Table 3. RANTES mediates PRV-induced lesions**

<table>
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<tr>
<th>Group</th>
<th>PRV</th>
<th>Animals With Lesions</th>
<th>Significance</th>
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<tr>
<td>Sham</td>
<td>Inactivated</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td>IsoAb</td>
<td>Active</td>
<td>9/12</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>α-RANTES</td>
<td>Active</td>
<td>4/12</td>
<td>P &lt; 0.05</td>
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PRV, pseudorabies virus.
flammatory conditions such as IC. This work and our previous studies elucidated the roles of TNF and RANTES by relying largely on genetic and histological approaches to the study of murine neurogenic cystitis. As a result, physiological changes associated with neurogenic cystitis are not completely characterized in this model. Altered cystometric findings have been reported in FIC and in some IC patients (8, 15, 39), so it is possible that murine neurogenic cystitis also results in altered bladder physiology.

Previously, human bladder detrusor smooth muscle cells were shown to secrete RANTES in response to TNF and IL-1β in culture (6). We observed an increase in RANTES immunoreactivity in the detrusor of PRV-infected mice that was absent in mice defective in TNF signaling (Fig. 3 and data not shown). These in vivo data thus support the previous in vitro findings of TNF-dependent RANTES expression in the detrusor. However, RANTES immunoreactivity was greater in the urothelium, and the timing of increased urothelial RANTES expression (PID 3 and 4) coincided with mast cell trafficking to the lamina propria (11). The differential expression of RANTES within the bladder suggests that a gradient of RANTES secretion is established, which may then promote mast cell trafficking toward the urothelium and accumulation in the lamina propria. Consistent with this possibility, lamina propria mast cell accumulation was associated with a corresponding decrease of mast cells in the lumen-proximal half of the detrusor, but mast cell counts remained stable in the lumen-distal layer of the detrusor (11). Thus we suggest a model where lumen-proximal detrusor mast cells traffic to the lamina propria along a gradient of RANTES, but lumen-distal mast cells are retained in the detrusor due to local RANTES secretion by smooth muscle cells.

Mast cell microlocalization within specific tissue structures is important in disease. For example, mast cells were found to be increased in the intestine of patients with irritable bowel syndrome (33, 47). Similarly, bladder biopsies from IC patients showed a correlation between lamina propria mast cells and patient symptoms, and urotheelial lesions were also associated with IC symptoms (29, 46). Since the PRV neurogenic cystitis model recapitulates both lamina propria mast cell accumulation and urothelial lesion formation in processes dependent on RANTES, we speculate that RANTES also plays a role in IC by recruiting mast cells to the lamina propria. RANTES is not specific for mast cells, however, and can mediate recruitment of diverse leukocytes. Indeed, increased leukocytic influx has been reported in bladder biopsies of some IC patients (40), and we observed a significant influx of non-PMN leukocytes into the bladder following PRV infection (11). While these findings raise the possible involvement of other leukocytes in contributing to patient symptoms or bladder pathogenesis in the PRV model, we found that neutralizing RANTES antibodies did not inhibit non-PMN leukocytic influx in the PRV model (Fig. 4B). Since anti-RANTES treatment blocked lamina propria mast cell accumulation and bladder dysfunction, these data support a dominant role for mast cells in the pathogenesis of neurogenic cystitis and further suggest a causative role for lamina propria mast cells in IC pathogenesis. Thus drawing similarities with asthma, colitis, and oral lichen planus, these data support an emerging picture of mast cell microlocalization in disease.

PRV-induced neurogenic cystitis is an acute model that recapitulates at least three findings associated with IC. Since anti-RANTES therapy blocks both the accumulation of lamina propria mast cells and the formation of urothelial lesions in the PRV model, findings that are also associated with IC symptoms, this suggests that anti-RANTES therapy may be efficacious for the management of IC patients with mast cell-associated disease. In a model of pulmonary granuloma formation, the efficacy of anti-RANTES therapy was dependent on the underlying T cell responses such that anti-RANTES treatment reduced pulmonary lesions in a Th1-polarized environment, but the same treatment exacerbated lesions in a Th2 environment (14). Although the potential involvement of T cell responses in IC is unknown, the incidence of Crohn’s disease is increased ~100-fold in IC patients relative to the general population (2). Since Crohn’s is often regarded as an aberrant Th1 response in genetically susceptible individuals (4, 36), it is possible that at least a subset of IC patients is similarly susceptible to aberrant Th1 responses and is thus an appropriate candidate for anti-RANTES therapy, especially in patients with bowel comorbidity. Indeed, anecdotal evidence indicates that Crohn’s patients suffering bladder comorbidity experience an improvement in bladder symptoms during when receiving anti-TNF therapy for their bowel symptoms (12).

In summary, we find that RANTES mediates a shift in mast cell microlocalization in neurogenic cystitis so that mast cells are juxtaposed with the urothelium. Blocking RANTES function with neutralizing antibodies inhibits altered mast cell microlocalization during disease, and anti-RANTES prevents the formation of urothelial lesions and stabilizes bladder barrier function. Since both lamina propria mast cells and urothelial lesions are associated with symptoms in IC, these findings suggest that RANTES represents a novel therapeutic target for the treatment of IC patients with mast cell-associated disease. Future studies will determine whether IC patient samples also contain elevated levels of RANTES.

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GRANTS

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