

Bio Cell  $\alpha$ -PD-1 ·  $\alpha$ -PD-L1 ·  $\alpha$ -CTLA-4 ·  $\alpha$ -CD20 ·  $\alpha$ -NK1.1 ·  $\alpha$ -IFNAR-1

DISCOVER MORE



## 5-Hydroxytryptamine Induces Mast Cell Adhesion and Migration

Nataliya M. Kushnir-Sukhov, Alasdair M. Gilfillan, John W. Coleman, Jared M. Brown, Sandra Bruening, Miklos Toth and Dean D. Metcalfe

This information is current as of August 9, 2022.

*J Immunol* 2006; 177:6422-6432; ;  
doi: 10.4049/jimmunol.177.9.6422  
<http://www.jimmunol.org/content/177/9/6422>

**References** This article **cites 55 articles**, 20 of which you can access for free at:  
<http://www.jimmunol.org/content/177/9/6422.full#ref-list-1>

**Why *The JI*? Submit online.**

- **Rapid Reviews! 30 days\*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*\*average*

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:  
<http://jimmunol.org/subscription>

**Permissions** Submit copyright permission requests at:  
<http://www.aai.org/About/Publications/JI/copyright.html>

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:  
<http://jimmunol.org/alerts>

*The Journal of Immunology* is published twice each month by  
The American Association of Immunologists, Inc.,  
1451 Rockville Pike, Suite 650, Rockville, MD 20852  
Copyright © 2006 by The American Association of  
Immunologists All rights reserved.  
Print ISSN: 0022-1767 Online ISSN: 1550-6606.



# 5-Hydroxytryptamine Induces Mast Cell Adhesion and Migration<sup>1</sup>

Nataliya M. Kushnir-Sukhov,<sup>2\*</sup> Alasdair M. Gilfillan,\* John W. Coleman,\* Jared M. Brown,\* Sandra Bruening,<sup>†</sup> Miklos Toth,<sup>†</sup> and Dean D. Metcalfe\*

The neurotransmitter serotonin (5-hydroxytryptamine (5-HT)) is implicated in enhancing inflammatory reactions of skin, lung, and gastrointestinal tract. To determine whether 5-HT acts, in part, through mast cells (MC), we first established that mouse bone marrow-derived MC (mBMMC) and human CD34<sup>+</sup>-derived MC (huMC) expressed mRNA for multiple 5-HT receptors. We next determined the effect of 5-HT on mouse and human MC degranulation, adhesion, and chemotaxis. We found no evidence that 5-HT degranulates MC or modulates IgE-dependent activation. 5-HT did induce mBMMC and huMC adherence to fibronectin; and immature and mature mBMMC and huMC migration. Chemotaxis was accompanied by actin polymerization. Using receptor antagonists and pertussis toxin, we identified 5-HT<sub>1A</sub> as the principal receptor mediating the effects of 5-HT on MC. mBMMC from the 5-HT<sub>1A</sub> receptor knockout mouse (5-HT<sub>1A</sub>R<sup>-/-</sup>) did not respond to 5-HT. 5-HT did induce accumulation of MC in the dermis of 5-HT<sub>1A</sub>R<sup>+/+</sup> mice, but not in 5-HT<sub>1A</sub>R<sup>-/-</sup> mice. These studies are the first to demonstrate an effect of 5-HT on MC. Furthermore, both mouse and human MC respond to 5-HT through the 5-HT<sub>1A</sub> receptor. Our data are consistent with the conclusion that 5-HT promotes inflammation by increasing MC at the site of tissue injury. *The Journal of Immunology*, 2006, 177: 6422–6432.

Mast cells (MC)<sup>3</sup> are known to increase in number at sites of inflammation (1, 2), where they are believed to influence vascular permeability, cell accumulation (3), and tissue-specific responses (3, 4). Of interest are reports that 5-hydroxytryptamine (5-HT; serotonin) is also increased at sites of inflammation such as in contact (5) and atopic (6–8) dermatitis; and in the blood and sputum of asthmatics (9, 10). In fact, platelets have been implicated in allergic inflammation in part through release of 5-HT (11, 12). Finally, 5-HT modifiers have been suggested as possible treatment for asthma (13). Both ketanserin, a blocking agent of 5-HT receptors, and tiapentine, which reduces the level of free serotonin in plasma by enhancing uptake in platelets, have been assessed in treatment of bronchial hyperresponsiveness (14) or bronchial asthma (9). Here, we thus studied the hypothesis that 5-HT acts in part by enhancing MC function in allergic inflammation.

5-HT receptors are G-protein-coupled membrane proteins with characteristic seven-transmembrane domains, and have been divided conventionally into seven subfamilies based on pharmacological properties, gene organization, and coupling to a second messenger. At least 14 subtypes encoded by distinct genes were

identified by molecular cloning techniques (15). The reported effects of 5-HT on inflammatory cells have generally been found to be mediated by 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>4</sub>, 5-HT<sub>6</sub>, or 5-HT<sub>7</sub> receptors (16–21). In initial experiments, we found both CD34<sup>+</sup>-derived human MC (huMC) and mouse bone marrow-derived MC (mBMMC) express a variety of 5-HT receptors, reinforcing the possibility that MC could respond to 5-HT. Subsequent experiments revealed that 5-HT can modulate both MC adhesion and migration. Pharmacological studies and protocols using mice lacking the 5-HT<sub>1A</sub> receptor showed that the predominant receptor mediating the effects of 5-HT on both mBMMC and huMC MC is 5-HT<sub>1A</sub>.

## Materials and Methods

### Animals used

Female wild-type C57BL/6 mice were purchased from The Jackson Laboratory; wild-type BALB/c mice were from Taconic Farms; 5-HT<sub>1A</sub> receptor knockout mice (that the point mutation results in a nonfunctional protein) (5-HT<sub>1A</sub>R<sup>-/-</sup>) and wild-type C57BL/6 littermate controls (5-HT<sub>1A</sub>R<sup>+/+</sup>) were maintained at the Department of Pharmacology, Weill Medical College of Cornell University (22). Mice were kept and sacrificed in accordance with National Institutes of Health and Weill Medical College of Cornell University animal care and use guidelines. The studies have been reviewed and approved by an appropriate institutional review committee.

### mBMMC culture

Cells, obtained from mouse femur, were cultured in RPMI 1640 medium supplemented with 10% FCS, glutamine (4 mM), sodium pyruvate (1 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), nonessential amino acids (1 mM), HEPES (25 mM), 2-ME (50 mM), and mouse rIL-3 (30 ng/ml; PeproTech) as described (23). Cell cultures were maintained at 37°C in 5% humidified CO<sub>2</sub> up to 6 wk. Immature cultures at 2 wk were studied for selective migration of immature mBMMC (24, 25); mature MC were obtained at 5–6 wk.

### huMC culture

huMC were cultured as described (26–28). In brief, peripheral blood CD34<sup>+</sup> progenitor cells were collected from healthy donors after informed consent and were processed by affinity column apheresis. CD34<sup>+</sup> cells

\*Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; and <sup>†</sup>Department of Pharmacology, Weill Medical College of Cornell University, New York, NY 10021

Received for publication May 4, 2006. Accepted for publication August 8, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> This work was supported by the National Institute of Allergy and Infectious Diseases-National Institutes of Health Intramural Research Grant Program.

<sup>2</sup> Address correspondence and reprint requests to Dr. Nataliya M. Kushnir-Sukhov, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Room 11C208, 10 Center Drive, MSC 1881, Bethesda, MD 20892-1881. E-mail address: nkushnir@niaid.nih.gov

<sup>3</sup> Abbreviations used in this paper: MC, mast cell; 5-HT, 5-hydroxytryptamine; huMC, CD34<sup>+</sup>-derived human MC; mBMMC, mouse bone marrow-derived MC; HSA, human serum albumin; β-hex, β-hexosaminidase; pAb, polyclonal Ab; SCF, stem cell factor; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; SERT, serotonin transporter.

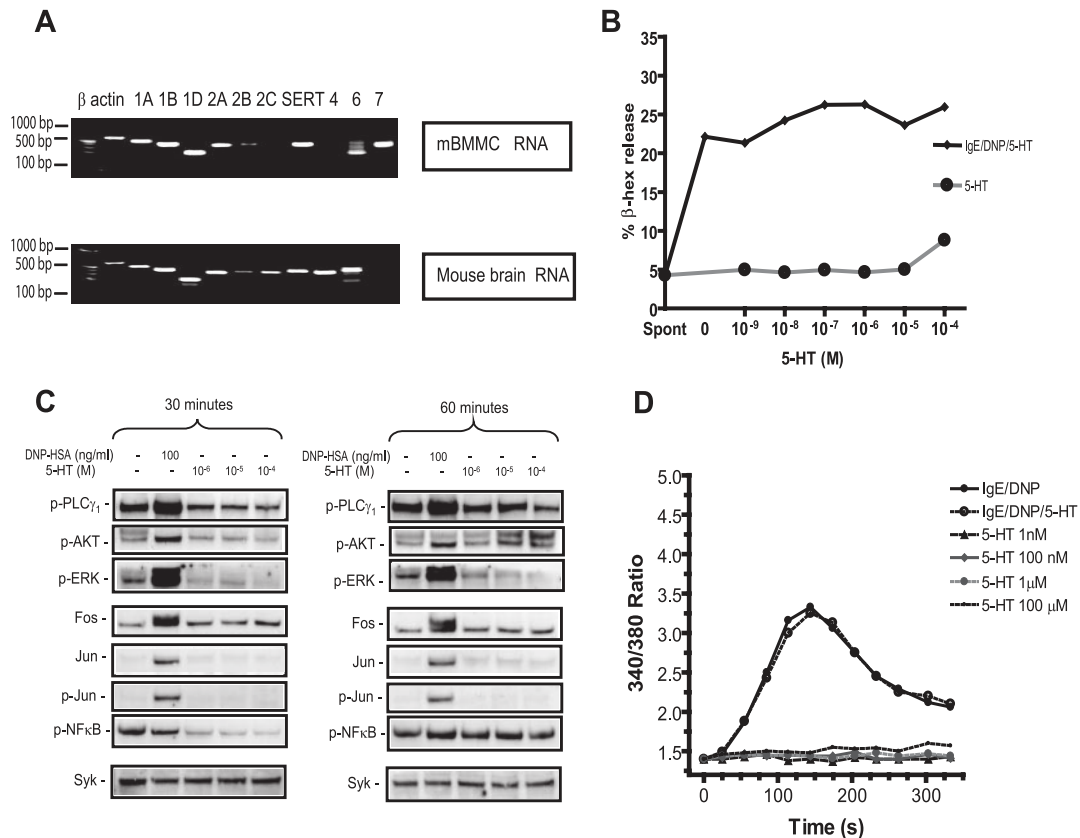
Table I. Primer sequences for 5-HT receptors

	Primer 1 (sense 5'- to 3'-)	Primer 2 (antisense 5'- to 3'-)	Size (bp)
<b>Murine</b>			
5-HT <sub>1A</sub>	TCTGTACCAGGTGCTCAACAAG	AGAGGAAGGTGCTCTTTGGAGT	643
5-HT <sub>1B</sub>	ATCAGCACCATGTACACCGTCA	GACTTGGTTTACGTTACACAGGA	555
5-HT <sub>1D</sub>	AGATGTCTGACTGCTGGTGAA	TGCGTCTAAGATGCTATCAGC	349
5-HT <sub>2A</sub>	AAGCCTCGAACTGGACAATTGATG	AAGATTTTCAGGAAGGCTTTGGTT	445
5-HT <sub>2B</sub>	CAGAAGACATGTGATCACCTGATC	TGTAATCTTGATGAATGCAGTAGCC	474
5-HT <sub>2C</sub>	GTTTGGCAGTTCGATATTCATA	GGCAATCTTCATGATGTAGTC	473
5-HT <sub>4</sub>	CCCATAATGCAAGGATGGAACA	GGAAGGCACGTCTGAAAGACTT	505
5-HT <sub>6</sub>	AGCCATGCTGAACGCGCTGTAT	CAAGGCCTTCTGCTATGCTTG	553
5-HT <sub>7</sub>	CCACTTCTTCTGCAACGCTTTC	GTGTTTGGAGCAGTCTCGAAAGG	481
SERT	ACATCTGGCGTTTTCCCTACAT	TTTTGACTCCCTTCCAGATGCT	477
5-HT <sub>1A</sub> <sup>R+/+</sup>	AGTGCAGGCAGGCATGGATATGTT	CCGATGAGATAGTTGGCAACATTCTGA	238
5-HT <sub>1A</sub> <sup>R-/-</sup>	CTTTACGGTATCGCCGCTCCCATTCC	TGCAGGATGGA CGAAGTGCAGCACA	400
<b>Human<sup>a</sup></b>			
5-HT <sub>1A</sub>	GCCGCGTGCCTCATCTCG	GCCGCGCCATCGTCACCTT	411
5-HT <sub>1B</sub>	CAGCGCCAAGGACTACATTTACCA	GAAGAAGGGCGGCAGCGAGATAGA	460
5-HT <sub>1E</sub>	CAAGAGGGCCGCGCTGATGAT	CTGCCTTCCGTTCCCTGGTGGTGCTA	461
5-HT <sub>2A</sub>	TGACGGCCATGATGTTTGTGAT	ACTCGCCATGATAACTTTGTCCT	359
5-HT <sub>2B</sub>	GGCCCTCCCACTTGTCTT	TAGGCGTTGAGGTGGCTTGT	416
5-HT <sub>2C</sub>	TGTGCCCGTCTGGATTTCTTTAGCTC	TTCCTCGGCCGATTCCTCTTCCG	449
5-HT <sub>3</sub>	GCCGCCCCCTCTTCTAT	GCAAAGTAGCCAGGCGATTTCTCTGGC	448/352
5-HT <sub>4</sub>	CTTCTACATCCCATTTCTCCTCTTCGG	TAGCGCTCATCATCACACCGCCGGCC	411
5-HT <sub>6</sub>	CCGCCGGCCATGCTGAAC	GCCCCAGCCACAAGGACAAAAG	342
5-HT <sub>7</sub>	GCGCTGGCCGACCTCTC	TCTTCTTGGCAGCCTTGTAATCT	436
SERT <sup>b</sup>	CATCTGGAAGGCGTCAAG	CGAAACGAAGCTCGTCATG	319
β-Actin <sup>c</sup>	ATCTGGCACCCACCTTCTACAATGAGCTGCG	CGTCATACTCTGCTTGCTGATCCACATCTGC	838

<sup>a</sup> Primers from Ref. 18.

<sup>b</sup> Primer from Ref. 56.

<sup>c</sup> Primer from Ref. 30.



**FIGURE 1.** mBMMC expression of 5-HT receptors: effect of 5-HT on IgE-mediated degranulation. *A*, mBMMC at 5 wk were analyzed for 5-HT receptor expression. Mouse brain total RNA was used as a positive control. Results are representative of three separate experiments using separate MC cultures initiated from different mice. *B*, mBMMCs were sensitized overnight with IgE anti-DNP and activated by DNP-HSA. 5-HT at concentrations noted was added to the wells simultaneously with Ag. β-Hex release was determined after 30 min. “Spont” equals spontaneous release; “0,” β-hex release when cells were activated with IgE/DNP-HSA alone. Results are representative of three experiments, each initiated from different mice. *C*, mBMMC ( $1 \times 10^6$  cells/condition) were activated through FcεRI or were treated with 5-HT and analyzed by Western blot for protein phosphorylation at 30 and 60 min. Line 1 represents levels of protein phosphorylation in unstimulated control cells. Syk was used for protein normalization. *D*, Ca<sup>2+</sup> flux was measured in mBMMC exposed over 300 s to stimuli indicated.

were cultured in StemPro-34 SFM (Invitrogen Life Technologies) in the presence of rIL-3 (first week only), rIL-6, and recombinant human stem cell factor (rhSCF) (PeproTech). All cultures were maintained up to 10 wk at 37°C and 5% CO<sub>2</sub>. One-half of the cytokine-supplemented medium was replaced weekly.

#### Cell activation and degranulation

mBMMC were sensitized with mouse monoclonal anti-DNP IgE (100 ng/ml; Sigma-Aldrich) and activated with DNP-human serum albumin (HSA) conjugate (100 ng/ml; Sigma-Aldrich). huMC were sensitized with biotinylated IgE (100 ng/ml) overnight and activated with streptavidin (100 ng/ml; Sigma-Aldrich). Degranulation was measured by granule-associated  $\beta$ -hexosaminidase ( $\beta$ -hex) release as described (29). 5-HT was prepared before each experiment.

#### RT-PCR

A total of 3–4  $\times 10^6$  cells was collected by centrifugation and processed with TRIzol (Invitrogen Life Technologies). The RNA layer was collected and transferred to an RNeasy column for further purification according to the manufacturer's instructions (Invitrogen Life Technologies). Five- to 7- $\mu$ g samples were also treated with 1 U of DNase to eliminate residual DNA contamination (30). One microgram of each sample was used in the RT-PCR (SuperScript III One-Step RT-PCR System; Invitrogen Life Technologies) with specific primers designed (Bio-Synthesis) for murine and human receptors (Table I).  $\beta$ -Actin was used as sample loading control. cDNA synthesis and PCR amplification were performed with a DNA Engine PTC-200 cyclor (Bio-Rad Laboratories) programmed with the following cycles: cDNA synthesis: 30 min at 55°C; denaturation: 2 min at 94°C; PCR amplification (30 cycles): 15 s at 94°C (denature), 30 s at 60°C (anneal); 2 min at 68°C (extend); final extension at 5 min at 68°C. Total mouse brain RNA (BD Biosciences) was used as a positive control for 5-HT receptor expression. For conformational analysis of the mutated nonfunctional insert in MC cultured from 5-HT<sub>1A</sub> R<sup>-/-</sup> and 5-HT<sub>1A</sub> R<sup>+/+</sup> mouse bone marrow, specific primers were used (Table I).

#### Cytokine measurement

mBMMC (1  $\times 10^5$ /100  $\mu$ l per well) were cultured for 24 h in 5-HT (10<sup>-3</sup>–10<sup>-6</sup> M) with or without prior activation through Fc $\epsilon$ RI. Supernatants were collected following centrifugation at 300  $\times g$  for 5 min. TNF- $\alpha$  (sensitivity, <0.18 pg/ml; R&D Systems) and IL-6 (sensitivity, <10 pg/ml; R&D Systems) were measured following R&D protocols.

#### Flow cytometry

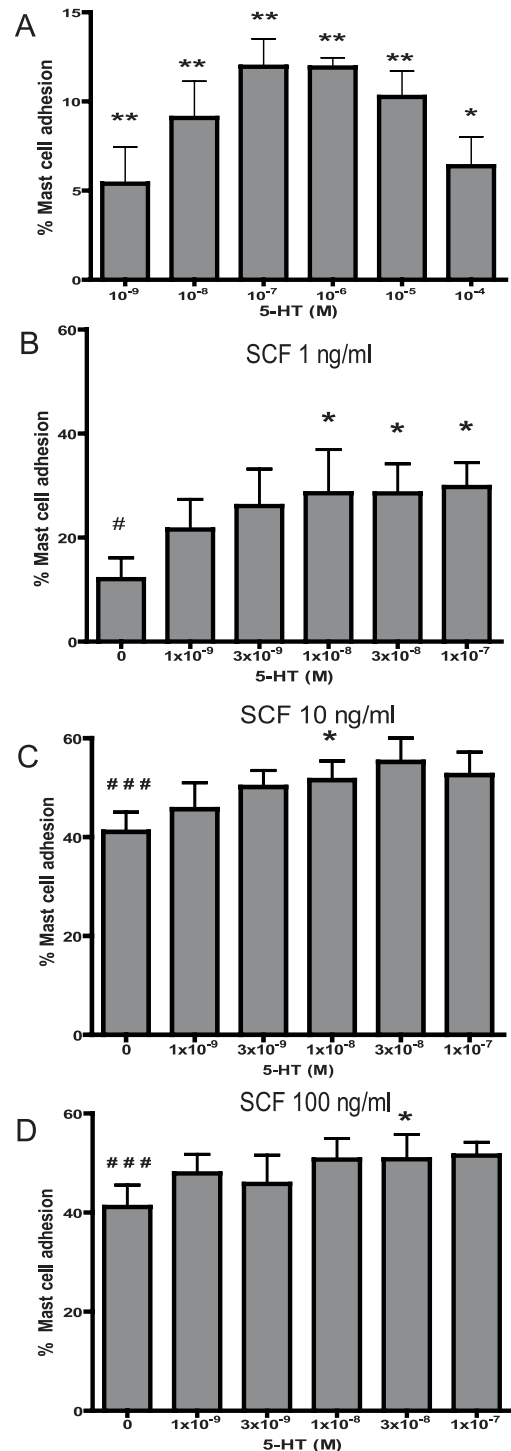
MC were identified by flow cytometry as double-positive cells for Fc $\epsilon$ RI and Kit. Propidium iodide staining was used to exclude the dead cell population. Fc $\epsilon$ RI/IgE, Kit (CD117), and adhesion molecule surface expression was analyzed as described (31). Staining for Fc $\epsilon$ RI was as follows: 5  $\times 10^5$  cells were sensitized for 2 h at 37°C with mouse monoclonal IgE-anti DNP (Sigma-Aldrich) at 100 ng/ml in RPMI 1640 medium, washed two times with PBS-BSA, and stained with DNP-FITC. Staining with anti-CD51, anti-CD11a, anti-CD54, and anti-CD49e (eBioscience) was performed at 4°C for 30 min. Cells were then washed, resuspended in PBS, and analyzed. Data were obtained on FACScan (BD Immunocytometry Systems) and analyzed with WinMDI 1.2 software (The Scripps Research Institute).

#### Actin polymerization (F-actin)

Cells were washed and resuspended in HEPES buffer with 1% BSA at 1  $\times 10^6$  cells/ml. Cells were kept at 37°C in a water bath and stimulated with 5-HT or the 5-HT<sub>1A</sub> receptor agonist *R*-(+)-8-(OH)-DPAT (Sigma-Aldrich) as indicated (see Fig. 6 legend). For inhibition experiments, 5-HT and the 5-HT<sub>1A</sub> receptor antagonist WAY100635 (Sigma-Aldrich) were both added to cells. Cells were fixed and permeabilized with 4% paraformaldehyde, 0.5% saponin, and 1.7  $\mu$ g/ml FITC-phalloidin conjugate (Invitrogen Life Technologies) for 10 min on ice, washed, and analyzed by flow cytometry (32). For immunostaining, 5  $\times 10^5$  cells were grown overnight on glass chamber slides, and then washed and stimulated with 5-HT, *R*-(+)-8-OH-DPAT or 5-HT with WAY100635. Staining for F-actin was performed as above. Cells were visualized and photographed using an Axiophot microscope (Zeiss) at  $\times 63$  (lens objective).

#### Signaling studies

Protein phosphorylation, in sensitized MC, was performed following Fc $\epsilon$ RI aggregation, addition of 5-HT or both (33). Proteins were separated on 4–12% NuPage Tris-Bis gels using NuPage MES-SDS running buffer



**FIGURE 2.** Effects of 5-HT on mBMMC adhesion to fibronectin in the presence or absence of SCF. mBMMC adhesion was analyzed using 96-well fibronectin-precoated plates. Cells ( $5 \times 10^4$ /well) were treated with various concentrations of 5-HT alone or in combination with SCF. Spontaneous adhesion was subtracted from all data sets. Effect of 5-HT alone (A) or in the presence of 1 ng/ml (B), 10 ng/ml (C), or 100 ng/ml (D) SCF. Values of *p* were calculated by two-way ANOVA; dose-dependent differences were assessed by Bonferroni's posthoc test or Student's *t* test. \*, *p* < 0.05; \*\*, *p* < 0.01, 5-HT vs spontaneous; #, <0.05; ###, <0.001, SCF vs spontaneous; \*, <0.05, 5-HT/SCF vs SCF alone. Data are represented as mean  $\pm$  SEM (*n* = 3–5).

(Invitrogen Life Technologies), transferred onto nitrocellulose membranes, and probed with the following Abs: anti-Fos polyclonal Ab (pAb) and anti-Jun pAb (Santa Cruz Biotechnology); anti-Syk mAb (4D10) from

UBI; anti-phospho-AKT (Ser(P)-473) pAb, anti-phospho-ERK (Thr(P)-202 and Tyr(P)-204), anti-phospho-Jun (Ser(P)-73) pAb, and anti-phospho-NF- $\kappa$ B (Ser(P)-536) pAb (Cell Signaling); and anti-phospho-PLC $\gamma$ 1 (Tyr(P)-783) pAb (Biosource). The membranes were then incubated with the appropriate secondary Ab conjugated to HRP (1:3000) (Amersham Biosciences). The immunoreactive bands were visualized using a Renaissance chemiluminescence kit (NEN) according to the manufacturer's protocol. Calcium flux was measured as described (34) using fura 2-AM as a probe.

### Adhesion assay

The adhesion assay was performed as described (35) with a slight modification. Serocluster 96-well flat-bottom microtiter culture plates (Costar) were coated overnight with 5  $\mu$ g/ml human fibronectin (Sigma-Aldrich) at 4°C, and then blocked with 4% BSA over 1 h in 37°C and washed. Cells at  $1 \times 10^6$ /ml were washed in RPMI 1640 and then incubated with 2  $\mu$ g/ml calcein-AM (Invitrogen Life Technologies) for 30 min at 37°C and resuspended in HEPES/0.01% BSA, followed by washing and incubation in RPMI 1640 for another 30 min. Labeled cells were plated at a final concentration of  $5 \times 10^4$  cells/well and stimulated with the 5-HT<sub>1A</sub> receptor agonist R-(+)-8-OH-DPAT, 5-HT, or 5-HT with or without pertussis toxin (Sigma-Aldrich) or antagonists WAY100635, the nonselective 5-HT<sub>2</sub> antagonist ketanserin, the 5-HT<sub>1B/1D/1A</sub> antagonist GR 127935, the 5-HT<sub>2B/2C</sub> antagonist SB 200646 (Sigma-Aldrich), or the 5-HT<sub>7</sub> antagonist sumatriptan (GlaxoSmithKline) at a concentration selected from the published reports (18, 36) and where pilot experiments had demonstrated a maximal effect. After incubation at 37°C for 60 min (for mBMMC) or 90 min (for huMC), nonadherent cells were carefully removed. Fluorescence was read on a TECAN GENios plate reader (ReTiSoft) at an excitation wavelength of 485 nm and an emission of 530 nm. The percentage of adherent cells was calculated as follows (35): fluorescence of adherent cells/fluorescence of total cells  $\times$  100 = % adherent cells.

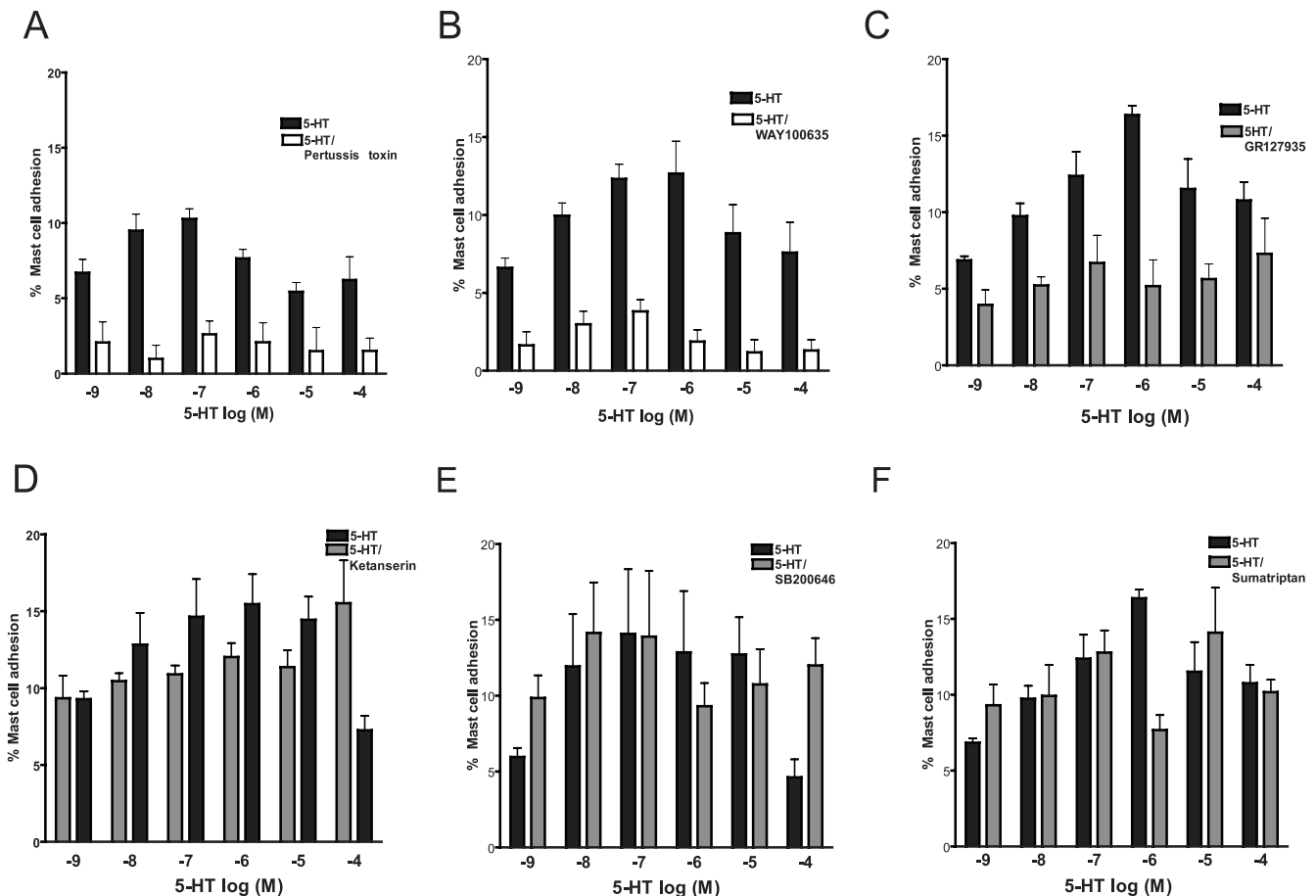
Direct fluorescence of negative controls (each chemical compound without cells) was assessed in parallel. Baseline (spontaneous) adhesion of cells to fibronectin was calculated for each separate experiment and subtracted from all data sets (average spontaneous mBMMC adhesion was 12–14%, and for huMC it was 8–10%).

### In vitro chemotaxis

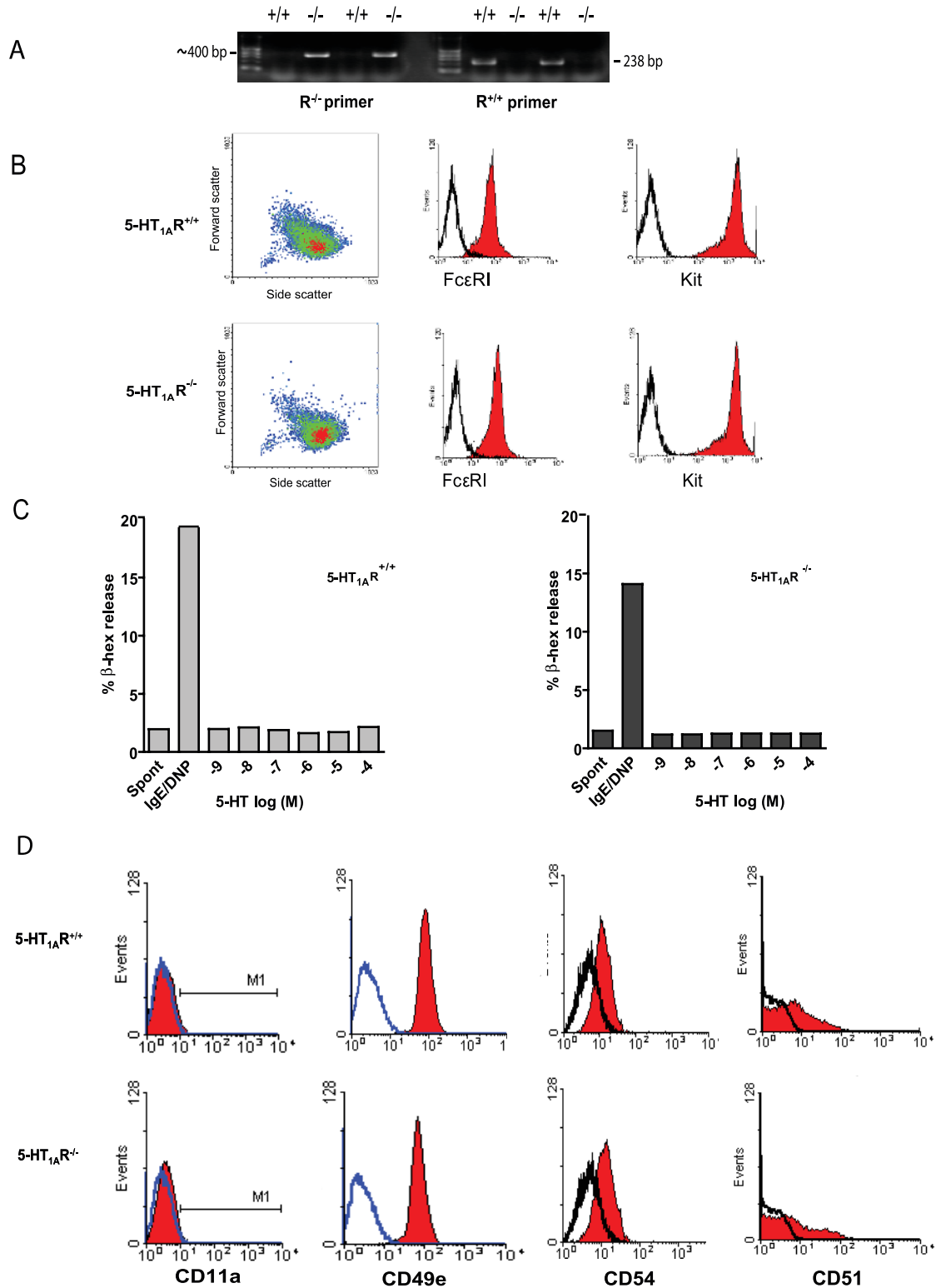
Chemotaxis was analyzed using a 96-well microchemotaxis system with 8- $\mu$ m pore size membranes (Neuroprobes) using direct fluorescence as described (37). Thus, cells were resuspended at  $1 \times 10^6$ /ml in HEPES plus 0.1% BSA, labeled with calcein-AM, and allowed to settle on the chemotaxis membranes for 10 min. Agents to be tested were diluted from stock solutions in HEPES/0.1% BSA and placed in the lower wells. Inhibitors, when tested, were added to the upper and lower wells at  $10^{-5}$  M before plate assembly. mBMMC were allowed to migrate for 60 min and huMC for 90 min, filters were removed, and migrated cells were counted based on direct fluorescence. A titration curve of fluorescence per cell was used for calculation of migrated cell number (linear regression in all experiments) (37). Human or murine rSCF (Sigma-Aldrich) at 10 ng/ml was used as a positive control (38). Chemokinesis was assessed by comparison of the migration of cells in the presence of the activation factor in both upper and lower wells. Baseline chemotaxis was subtracted ( $\sim$ 4000 for mBMMC and 2000 for huMC).

### Immature MC chemotaxis

Two-week-old immature cultured mBMMC as described (24) were studied for chemotaxis. Cells were plated without fluorescent staining on the chemotaxis filters and allowed to settle for 10 min. The filter was assembled with the lower plate filled with chemotactic agent(s) and placed at 37°C for 60 or 90 min. The lower well content (30 ml of PBS/0.1% BSA with transmigrated cells) was collected and stained with FITC-antiGr-1 (RB6-8C5) (BD Biosciences) and PE-CD117 (7B8) (BD Biosciences) for 30 min



**FIGURE 3.** Effect of pertussis toxin and specific 5-HT antagonists on mBMMC adhesion. mBMMC adhesion was examined in cells pretreated with pertussis toxin (A), the selective 5-HT<sub>1A</sub> antagonist WAY100635 (B), the 5-HT<sub>1B/1D/1A</sub> antagonist GR 127935 (C), the nonselective 5-HT<sub>2</sub> antagonist ketanserin (D), the 5-HT<sub>2B/2C</sub> antagonist SB 200646 (E), and the 5-HT<sub>7</sub> antagonist sumatriptan (F). In B–F, antagonists were added 3 min before the addition of 5-HT. Data as shown are presented as the mean  $\pm$  SEM of two to three experiments, each performed in triplicate on separately cultured mBMMC. Using two-way paired ANOVA, the inhibition observed in A–C was significant ( $p < 0.001$ ).

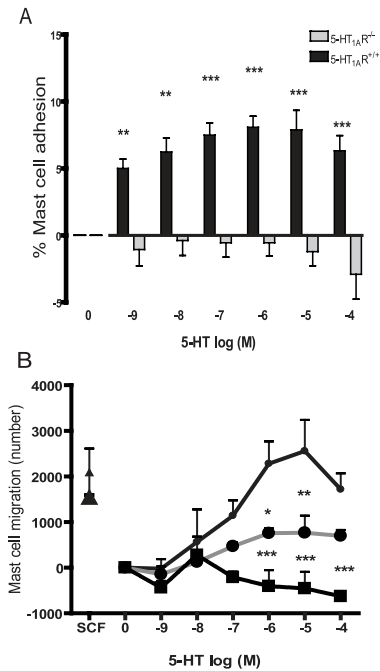


**FIGURE 4.** Characteristics of mBMMC cultured from 5-HT<sub>1A</sub>R<sup>-/-</sup> and 5-HT<sub>1A</sub>R<sup>+/+</sup> mice. **A**, The presence of the 5-HT<sub>1A</sub>R<sup>-/-</sup> nonfunctional insert is demonstrated in 5-HT<sub>1A</sub>R<sup>-/-</sup> cultured mBMMC, whereas the WT sequence shown in 5-HT<sub>1A</sub>R<sup>+/+</sup> mBMMC. **B**, Flow cytometry demonstrates that both 5-HT<sub>1A</sub>R<sup>+/+</sup> and 5-HT<sub>1A</sub>R<sup>-/-</sup> mBMMC similarly express FcεRI and Kit (CD117). **C**, 5-HT<sub>1A</sub>R<sup>+/+</sup> and 5-HT<sub>1A</sub>R<sup>-/-</sup> mBMMC are shown to similarly degranulate to IgE-mediated signals and do not respond to 5-HT. **D**, Adhesion molecules involved in MC adhesion to fibronectin were similarly expressed in 5-HT<sub>1A</sub>R<sup>-/-</sup> and 5-HT<sub>1A</sub>R<sup>+/+</sup> mice. Data presented in all panels are representative of at least three separately performed experiments.

on ice. Propidium iodide (BD Biosciences) was added 5 min before analysis. Total cell number was determined for each sample. Migrated immature MC were defined as cells positive for CD117 and negative for Gr-1 (granulocyte marker).

#### Migration of MC in vivo

Wild-type mice, between 2 and 2.5 mo of age, were used for skin injections (National Institutes of Health protocol ASP LAD 2). Bilateral flanks were

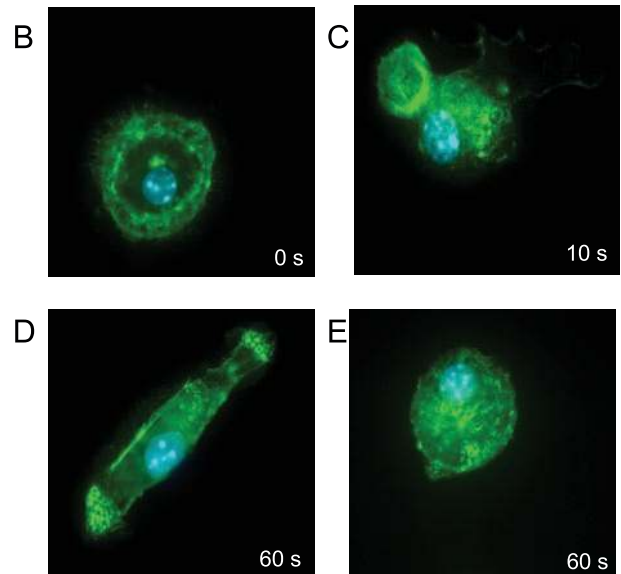
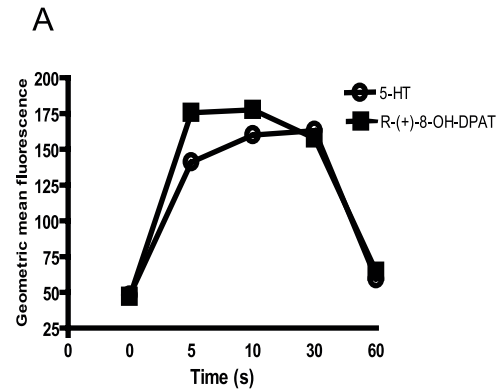


**FIGURE 5.** Comparison of adhesion and chemotaxis to 5-HT in mBMMC cultured from 5-HT<sub>1A</sub>R<sup>-/-</sup> and 5-HT<sub>1A</sub>R<sup>+/+</sup> mice. *A*, Adhesion of mBMMC cultured from 5-HT<sub>1A</sub>R<sup>-/-</sup> and 5-HT<sub>1A</sub>R<sup>+/+</sup> mice in the presence of 5-HT. Data are presented as a mean  $\pm$  SEM of six experiments performed in triplicate. Two-way ANOVA was applied to these data to compare differences in adhesion of 5-HT<sub>1A</sub>R<sup>-/-</sup> and 5-HT<sub>1A</sub>R<sup>+/+</sup> mBMMC ( $p < 0.001$ ) followed by Bonferroni's posthoc test to compare values within each concentration. *B*, Comparison of 5-HT<sub>1A</sub>R<sup>-/-</sup> and 5-HT<sub>1A</sub>R<sup>+/+</sup> mBMMC migration to SCF ( $\blacktriangle$ , 5-HT<sub>1A</sub>R<sup>+/+</sup>;  $\triangle$ , 5-HT<sub>1A</sub>R<sup>-/-</sup>) or 5-HT alone ( $\bullet$ , 5-HT<sub>1A</sub>R<sup>+/+</sup>;  $\circ$ , 5-HT<sub>1A</sub>R<sup>-/-</sup>) or 5-HT<sub>1A</sub>R<sup>+/+</sup> in the presence of WAY100635 ( $\square$ ). Two-way ANOVA was used to compare effect of 5-HT on 5-HT<sub>1A</sub>R<sup>-/-</sup>, 5-HT<sub>1A</sub>R<sup>+/+</sup>, and 5-HT<sub>1A</sub>R<sup>+/+</sup> mBMMC exposed to inhibitor ( $p < 0.01$ ). Bonferroni's multiple comparison test compared variables within each concentration. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .

shaved and washed with alcohol. 5-HT creatinine sulfate complex (Sigma-Aldrich) was diluted in sterile PBS to a final concentration of 10  $\mu$ M. Fifty microliters of 5-HT or vehicle (PBS alone) was injected (left flank and right flank). Two schedules were used as follows: 1 day with three injections 3 h apart; 2 days with three injections 4 h apart on each day. Mice were sacrificed 1 h after the last injection. Skin biopsies (10  $\times$  10 mm) were obtained at the 5-HT injection site and from the contralateral control site, which had received only PBS. Skin specimens were transferred to Carnoy's fixative (60% ethanol, 30% chloroform, and 10% acetic acid) for 12 h at 4C. The presence of tissue MC was assessed in 3- $\mu$ m, paraffin-embedded, toluidine blue O/eosin-stained sections. MC were counted by light microscopy per injection site (three slides per specimen from three different animals per each condition) using a 10  $\times$  10  $\mu$ m grid at  $\times$ 10 magnification, and average MC number per square millimeter was determined. Sections were number-coded so that the observer was blinded to the identity of individual specimens. Tissue digital photography of representative slides (one per group of three animals, each injected in contralateral sites with vehicle or 5-HT) was performed on Axiophot Ziex microscope at  $\times$ 10 (lens objective).

#### Statistical analysis

One-way or two-way mixed factorial ANOVA followed by the Bonferroni's post hoc test or Dunnett's multiple comparison test were used to determine statistical significance of response variations. Paired Student's *t* test ANOVA was used to assess dose-dependent effect on *in vivo* model. Differences were accepted to be statistically significant at  $p < 0.05$  (\* or #),  $p < 0.01$  (\*\* or ##), and  $p < 0.001$  (\*\*\*) or ###).



**FIGURE 6.** 5-HT induced actin polymerization in mBMMC. *A*, mBMMC were stimulated with 10<sup>-5</sup> M 5-HT or R-(+)-8-OH-DPAT for 10 and 60 s on multichamber glass slides, after which cells were stained with PE-conjugated phalloidin toxin bound to polymerized F-actin. Note the x-axis is not a linear scale. *B–D*, Fluorescent confocal microscopy of an mBMMC before 5-HT exposure (*B*), or 10 s after exposure to 5-HT (*C*), or 60 s after exposure of 5-HT (*D*). *E*, mBMMC 60 s after exposure of 5-HT and WAY100635.

## Results

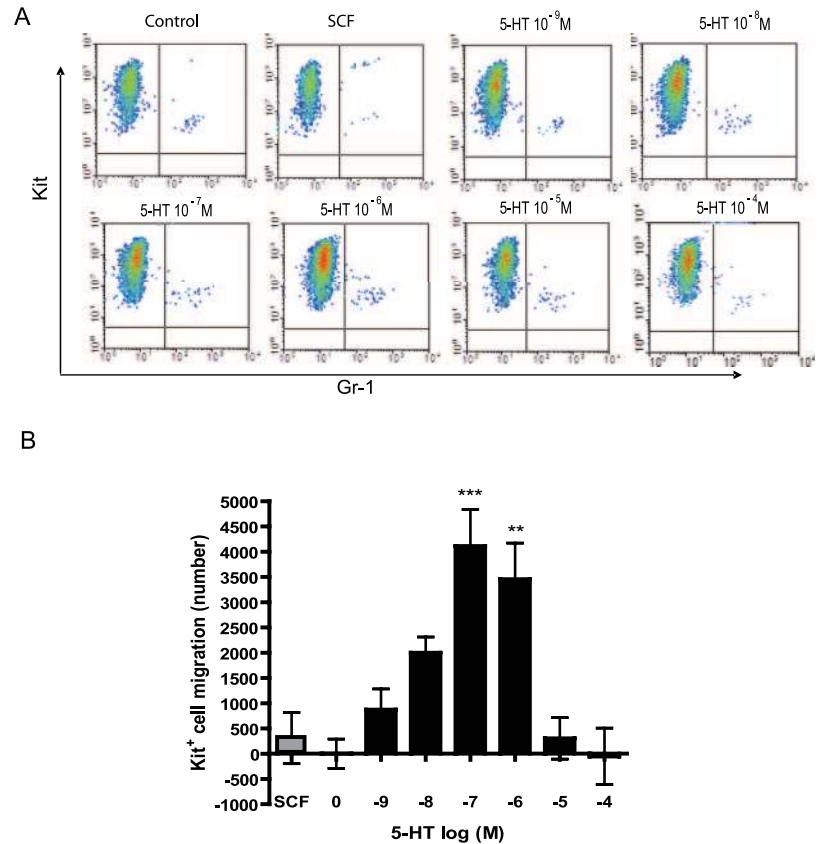
### mBMMC express 5-HT receptors

As the first step in the analysis of whether 5-HT affects mBMMC function, expression of mRNA for 5-HT receptors was determined (Fig. 1*A*). mBMMC were found to express mRNA for the 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> receptors as well as for serotonin transporter (SERT) (39). No mRNA was detected for 5-HT<sub>2C</sub> and 5-HT<sub>4</sub> receptors in mBMMC. Thus, mBMMC expressed mRNA for receptors for 5-HT that have been reported to mediate the effect of 5-HT on other cells of hemopoietic origin (16–21).

### 5-HT does not affect MC degranulation and cytokine release

Because MC are known to degranulate in inflammation in the dermis where 5-HT is also found to be increased (6, 40), we initially determined whether 5-HT directly induces MC degranulation or alters IgE-mediated degranulation. As Fig. 1*B* shows, 5-HT neither induced mBMMC degranulation, nor did it alter IgE-mediated degranulation. In data not shown, production of either TNF- $\alpha$  or IL-6 was similarly not altered after culture of mBMMC in 5-HT

**FIGURE 7.** Chemotaxis of immature mBMMC to 5-HT. Migration of immature mBMMC across a microchemotaxis filter was monitored by gating on Kit<sup>+</sup>Gr1<sup>-</sup> cells. **A**, Cells that have migrated and are Kit<sup>+</sup>Gr1<sup>-</sup> appear in the *upper left quadrant*. The numbers of migrated cells captured as shown by color density increase when 5-HT is present in the lower chamber, compared with background migration or in the presence of SCF, which induced minimal migration. **B**, Data are presented as Kit<sup>+</sup> cell migration determined from a standard curve of fluorescence per cell. Results of three separate experiments, each done in triplicate, are presented as mean  $\pm$  SEM. One-way ANOVA ( $p < 0.001$ ) with Bonferroni's posthoc test was used for analysis. \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .



( $10^{-3}$ – $10^{-6}$  M) for 24 h or after Fc $\epsilon$ RI-mediated degranulation followed by culture for 24 h in 5-HT. To confirm these observations, we next examined levels of phosphorylation of proteins activated in MC following IgE-mediated degranulation (see Fig. 1C, second column at 30 and 60 min) and following exposure to 5-HT alone (columns 3, 4, and 5). The protein phosphorylation induced by Fc $\epsilon$ RI aggregation was not observed after exposure of MC to 5-HT. Similarly, 5-HT did not affect Ca<sup>2+</sup> flux in MC either when added alone or when added at the time of activation of MC through Fc $\epsilon$ RI (Fig. 1D). These data are consistent with the conclusion that 5-HT does not induce nor influence MC degranulation or cytokine release.

#### 5-HT induces adhesion of MC to fibronectin

We next examined whether 5-HT enhances mBMMC adhesion to fibronectin, a component of tissue matrix. As Fig. 2A shows, 5-HT induced mBMMC adhesion to fibronectin in a concentration-dependent manner. Maximal adhesion was observed at  $10^{-7}$ – $10^{-6}$  M 5-HT. Adhesion induced by 5-HT was comparable to that induced by SCF alone at 1 ng/ml (Fig. 2B, first column), a known adhesion promotion factor for MC. When SCF at 1 ng/ml with increasing concentrations of 5-HT were simultaneously added, there was an additive effect on MC adhesion. SCF at 10 or 100 ng/ml resulted in a higher level of adhesion than at 1 ng/ml, and 5-HT had less effect in combination (Fig. 2, C and D), presumably because at these concentrations of SCF, adhesion is near maximal. These data show that 5-HT alone induces MC adhesion to fibronectin, and this effect is additive at lower concentrations of SCF.

#### Pertussis toxin blocks 5-HT-induced adhesion of MC to fibronectin

5-HT receptors are members of the G-protein-coupled receptor family. G $\alpha_i$  subunit, which is pertussis toxin sensitive (15), binds

only to 5-HT receptors 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, and 5-HT<sub>1D</sub>. As can be seen in Fig. 3A, pertussis toxin preincubation abolished 5-HT-induced adhesion of mBMMC to fibronectin, suggesting that 5-HT-induced adhesion is through the 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, or 5-HT<sub>1D</sub>.

#### A 5-HT<sub>1A</sub> receptor antagonist inhibits 5-HT induced adhesion of MC to fibronectin

We next examined the effect of WAY100635 (Fig. 3B), GR127935 (C), ketanserin (D), SB200646 (E), and sumatriptan (F) on 5-HT-induced mBMMC adhesion. We found that WAY100635 at 10  $\mu$ M completely blocked and GR127935 at 10  $\mu$ M significantly inhibited 5-HT induced adhesion (see Fig. 3). Other antagonists were less effective in blocking 5-HT induced adhesion. These data are consistent with the conclusion that 5-HT, acting through the 5-HT<sub>1A</sub> receptor, mediates increased adhesion to fibronectin.

#### 5-HT<sub>1A</sub>R<sup>-/-</sup>-derived mBMMC exhibit defective 5-HT-mediated adhesion

To confirm that 5-HT, acting via the 5-HT<sub>1A</sub> receptor, is mediating MC adhesion to fibronectin, we next examined mBMMC derived from 5-HT<sub>1A</sub> receptor knockout mice (5-HT<sub>1A</sub>R<sup>-/-</sup>). We first confirmed the presence of the 5-HT<sub>1A</sub>R mutation in mBMMC derived from 5-HT<sub>1A</sub>R<sup>-/-</sup> by RT-PCR with specific primers. As expected, total MC mRNA derived from 5-HT<sub>1A</sub>R<sup>-/-</sup> MC samples were positive with R<sup>-/-</sup> primers but not with R<sup>+/+</sup> specific primers, whereas MC mRNA derived from 5-HT<sub>1A</sub>R<sup>+/+</sup> mice were positive only for the R<sup>+/+</sup> specific primers (Fig. 4A). Then, we compared the cellular phenotype of 5-HT<sub>1A</sub>R<sup>-/-</sup> mBMMC with 5-HT<sub>1A</sub>R<sup>+/+</sup> mBMMC. No significant differences in expression of Kit and Fc $\epsilon$ RI were found between cultures (Fig. 4B). Similarly, there was no significant difference in  $\beta$ -hex release in response to Fc $\epsilon$ RI aggregation or to 5-HT stimulation between mBMMC from 5-HT<sub>1A</sub>R<sup>+/+</sup> and 5-HT<sub>1A</sub>R<sup>-/-</sup> mice (Fig. 4C). 5-HT<sub>1A</sub>R<sup>+/+</sup> mBMMC had similar levels of cell surface markers



involved in adhesion to fibronectin, including CD11a, CD49e, CD51, and CD54 (Fig. 4D). Thus, we found no difference in 5-HT<sub>1A</sub>R<sup>+/+</sup> and 5-HT<sub>1A</sub>R<sup>-/-</sup> MC in terms of Kit and FcεRI expression, degranulation, or expression of adhesion molecules. When 5-HT<sub>1A</sub>R<sup>-/-</sup> mBMMC were exposed to 5-HT, no 5-HT-induced adhesion was observed, whereas 5-HT<sub>1A</sub>R<sup>+/+</sup> mBMMC did adhere to fibronectin (Fig. 5A). Taken together, these observations establish that the 5-HT<sub>1A</sub> receptor is the principal receptor responsible for mediating the effect of 5-HT on mBMMC adhesion.

#### mBMMC chemotaxis to 5-HT is mediated through 5-HT<sub>1A</sub> receptor

We then examined whether mBMMC would migrate to 5-HT. As can be seen in Fig. 5B, 5-HT<sub>1A</sub>R<sup>+/+</sup> mBMMC exhibited dose-dependent chemotaxis to 5-HT. When 5-HT<sub>1A</sub>R<sup>+/+</sup> MC were exposed to 5-HT in the presence of WAY100635, chemotaxis was abolished. 5-HT<sub>1A</sub>R<sup>-/-</sup> MC demonstrated defective chemotaxis in response to 5-HT. These findings show that mBMMC migrate to 5-HT and that the action of 5-HT on mBMMC migration is mediated by the 5-HT<sub>1A</sub> receptor.

#### 5-HT promotes actin polymerization (F-actin) in mBMMC

We next examined actin polymerization, which accompanies signals that induce cell migration. Flow cytometry was used in the analysis of actin polymerization as assessed by monitoring fluorescence of bound phalloidin toxin in mBMMC following exposure to 5-HT (Fig. 6A). Baseline fluorescence ( $G_{\text{mean}} = 47.76$ ) was tripled when mBMMC were exposed to 5-HT ( $G_{\text{mean}} = 150$ ) or to the 5-HT<sub>1A</sub> receptor selective agonist R-(+)-8-OH-DPAT ( $G_{\text{mean}} = 175$ ) at 1 μM. Polymerization was observed within 5 s. Actin polymerization was also detected in mBMMC grown on glass chamber slides. Leading edges could be observed as early as 10 s, followed by cytoskeletal reorganization and cell shape changes, including cell elongation and membrane ruffling (Fig. 6, B–E). Similar observations were made in other cell types exposed to chemotactic stimuli consistent with our data that 5-HT acting through 5-HT<sub>1A</sub> receptor signals mBMMC movement (32).

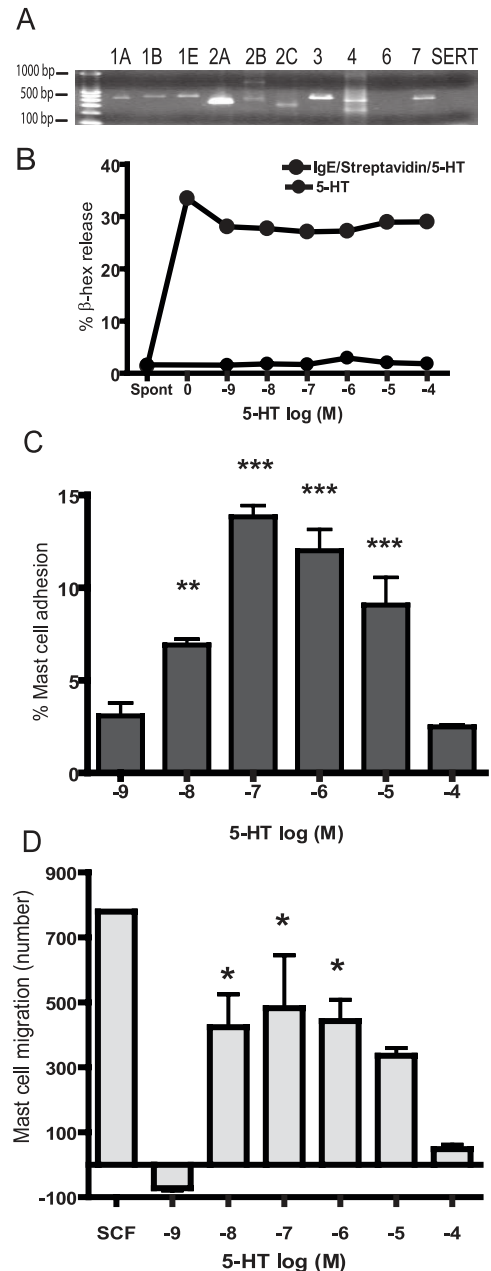
#### Immature MC migrate to 5-HT

It has recently been reported that immature mBMMC migrate to leukotriene B<sub>4</sub> (LTB<sub>4</sub>) but not to chemokines, indicating that LTB<sub>4</sub> is important in regulating tissue MC numbers (24). To determine whether 5-HT could similarly attract immature MC, we examined immature MC for their response to 5-HT. We monitored movement of immature MC across a membrane by determining the accumulation of immature Kit<sup>+</sup> mBMMC in the lower chamber. Gr-1<sup>+</sup> cells that thus meet the definition of granulocytes (remaining in 2-wk MC cultures) were excluded (Fig. 7A). The numbers of migrated Kit<sup>+</sup>Gr-1<sup>-</sup> cells were monitored, and we observed a significant dose-dependent increase (Fig. 7B). Maximal migration of immature MC was observed using 5-HT in concentrations between  $1 \times 10^{-7}$  and  $1 \times 10^{-6}$  M. Migration of immature mBMMC induced by 10 ng/ml SCF was significantly less than 5-HT-induced migration, consistent with the report that SCF does not play a major role in chemotaxis of immature mBMMC (24). Thus, as is reported for LTB<sub>4</sub>, 5-HT is a potent chemoattractant for immature mBMMC.

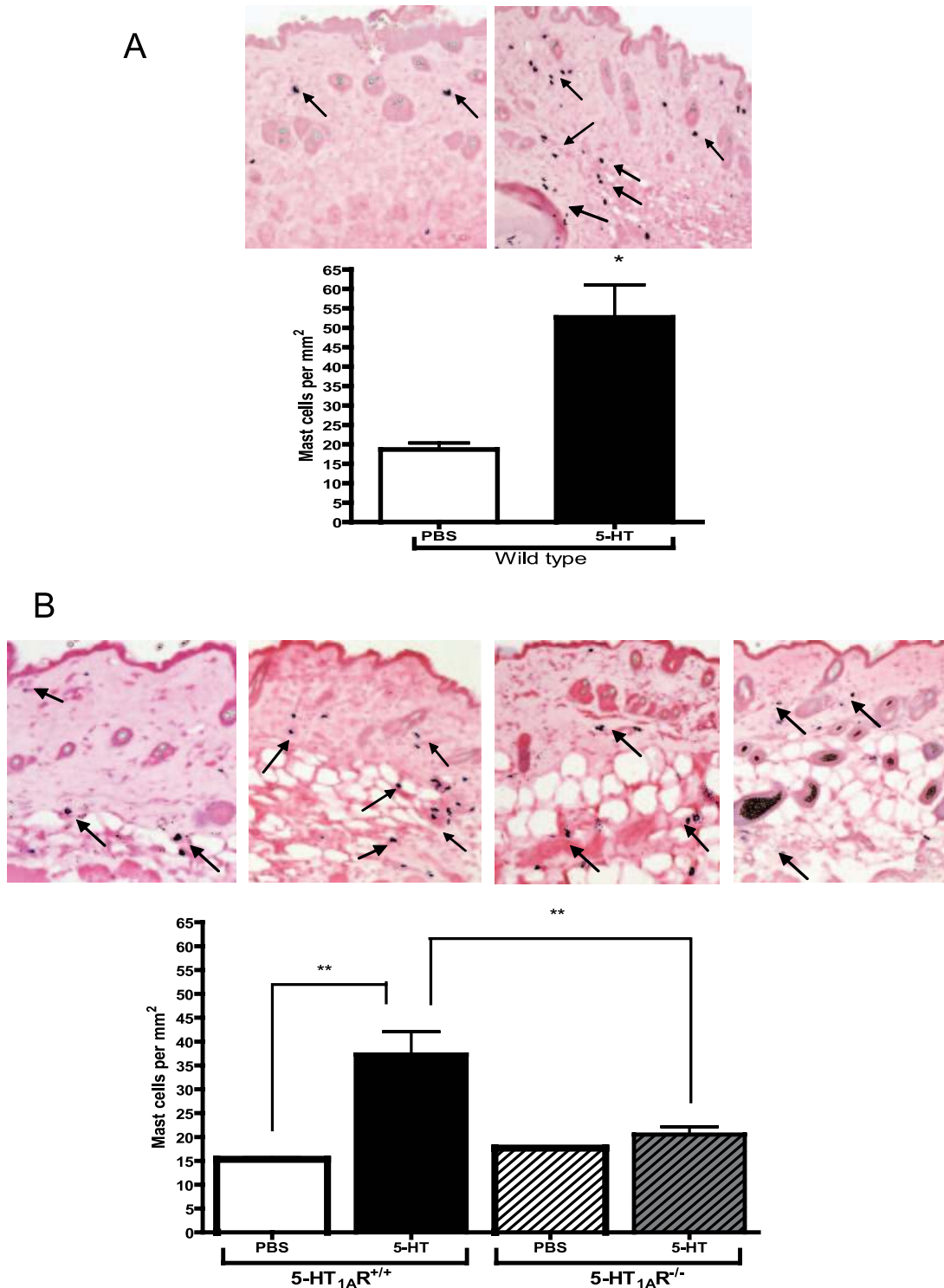
#### Effect of 5-HT on human MC adhesion and chemotaxis

We next investigated whether, similar to mBMMC, huMC are also affected by 5-HT. We first determined that cultured 8-wk-old

huMC express mRNA for the 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1E</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub>, and 5-HT<sub>7</sub> receptors (Fig. 8A). As with mBMMC, huMC released β-hex following FcεRI aggregation, but 5-HT neither altered FcεRI-mediated degranulation nor induced degranulation (Fig. 8B). However, huMC showed increased adhesion to fibronectin when exposed to 5-HT (Fig. 8C)



**FIGURE 8.** HuMC adhesion and migration induced by 5-HT. *A*, huMC express message for multiple 5-HT receptors. Data presented are representative of three separate experiments, each from cultured huMC from a separate donor. *B*, huMC degranulate to IgE-mediated signals, but not to 5-HT alone. 5-HT does not alter IgE-mediated degranulation. “0,” β-hex release when cells were activated by FcεRI aggregation only. Representative of two independent experiments. *C*, 5-HT induced huMC adhesion. SCF-induced adhesion was used as a positive control (on average 40%);  $n = 2$ ; mean  $\pm$  SEM. One-way ANOVA ( $p < 0.001$ ) with Bonferroni’s posthoc test was used to analyze the data. \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ . *D*, huMC migration to 5-HT was compared with migration induced by SCF. One-way ANOVA ( $p < 0.01$ ) was followed by Bonferroni’s ANOVA. \*,  $p < 0.05$ .



**FIGURE 9.** MC migration to the site of intradermal 5-HT injection. *A*, Microphotographs show representative examples of MC numbers at the sites of vehicle or 5-HT injections over 48 h. *Lower panels*, MC number per square millimeter in the dermis of BALB/c mice at the site of a control injection of PBS or 5-HT diluted in PBS,  $n = 6$  (six mice, three sections per mouse), mean  $\pm$  SEM. Student's  $t$  test was used to compare samples. \*,  $p < 0.05$ . *B*, Comparison of MC number per square millimeter after injection of 5-HT over 48 h into the dermis of 5-HT<sub>1A</sub>R<sup>-/-</sup> and 5-HT<sub>1A</sub>R<sup>+/+</sup> mice,  $n = 3$  (three mice, three sections per mouse), mean  $\pm$  SEM. Statistical significance was measured by Student's  $t$  test. \*\*,  $p < 0.01$ .

and migrated to 5-HT (*D*). 5-HT-induced migration was abolished in the presence of WAY100635 consistent with the conclusion that it is 5-HT<sub>1A</sub> dependent (data not shown).

#### 5-HT-dependent MC accumulation in vivo

Because 5-HT induces MC migration and adhesion in vitro, we asked whether 5-HT could lead to MC accumulation in vivo. To

examine this possibility, 5-HT was injected into mouse skin, and MC number was determined. Injection of 5-HT into the dermis of wild-type mice led to a significant increase in the accumulation of MC at the site of 5-HT injections after 48 h (Fig. 9*A*) and 7 h (*B*). However, this effect was not observed in the 5-HT<sub>1A</sub>R<sup>-/-</sup> mouse (Fig. 9*B*), indicating that 5-HT affects MC migration in vivo via a 5-HT<sub>1A</sub> receptor-dependent mechanism.

## Discussion

The role of 5-HT in MC function has not yet been investigated, and here we demonstrate that both huMC and mBMMC adhesion and migration respond to 5-HT. We also demonstrate that these biological functions are mediated by the 5-HT<sub>1A</sub> receptor. The preservation of responses to 5-HT from rodent to human supports the notion that these pathways are evolutionally consequential. Not all functions of mouse MC are conserved in human MC. For instance, whereas mBMMC will proliferate in the presence of IL-3 or SCF, huMC only show significant proliferation to SCF (41, 42). Note we found no evidence that 5-HT either could induce MC degranulation or cytokine production, nor alter the consequences of IgE-mediated degranulation.

MC of rodent origin (43) and human origin (44) both synthesize and concentrate 5-HT. Indeed, the uptake of [<sup>3</sup>H]5-HT in rodent MC in culture with its subsequent release following IgE-mediated degranulation is commonly used in studies of MC function (45–47). The possibility thus exists of an autocrine loop where 5-HT, released following MC activation, enhances the subsequent events by increasing MC at the site of inflammation.

5-HT is reported to influence behavior of inflammatory cells, inducing up-regulation of phagocytosis by macrophages (16), protecting NK cells from oxidative stress (17), promoting mitogen-activated T and B cell survival (21, 48), inducing migration of eosinophils (19), and modulating cytokine release from dendritic cells (18). The principal receptor involved in this process, when determined, has been identified as the 5-HT<sub>1A</sub> receptor (17, 21). Several lines of evidence support the notion that MC respond to 5-HT through the 5-HT<sub>1A</sub> receptor including the pertussis toxin-dependent inhibition of adhesion, the ability to disrupt adhesion and chemotaxis with the 5-HT<sub>1A</sub> receptor antagonist WAY100635, and the lack of response of MC derived from 5-HT<sub>1A</sub>R<sup>-/-</sup> mouse. 5-HT<sub>2A</sub> is predominantly expressed in human MC; however, the evidence is compelling that 5-HT<sub>2A</sub> is not the predominant 5-HT receptor that allows the human MC to respond to 5-HT in adhesion and chemotaxis. This is because in human MC pertussis toxin significantly abrogates the response to 5-HT, and pertussis toxin is known to inhibit 5-HT<sub>1A</sub> (G<sub>i</sub>-coupled), but not 5-HT<sub>2A</sub> (G<sub>q</sub>, G<sub>11</sub>-coupled) responses. This observation, coupled with the inhibition of 5-HT<sub>1A</sub> with specific antagonist allows us to feel comfortable with the conclusion that 5-HT<sub>1A</sub> is the predominant receptor involved in human MC adhesion and chemotaxis to 5-HT.

The possible in vivo relevance of 5-HT-induced MC adhesion and migration is illustrated by the increase of MC at sites of 5-HT injection in mouse dermis. MC accumulation did not occur in 5-HT<sub>1A</sub>R<sup>-/-</sup> mice, demonstrating that the 5-HT<sub>1A</sub> receptor is also involved in responses in vivo. However, we cannot exclude the possibility that the effect of 5-HT in vivo is working indirect on MC. For instance, 5-HT could act on another cell in mouse dermis, which in turn could release a chemotactic factor attracting MC.

The origin of MC that accumulate at the sites of 5-HT injection is not known. It is possible that MC near the site of injection are attracted to the injection site over a few hours. MC progenitors additionally might be recruited from the blood if the level of 5-HT remains persistently elevated at the tissue site. Although not confirmed in vivo, in vitro experiments strongly suggest that 5-HT recruits MC progenitors, much like LTB<sub>4</sub> (24), and may be implicated in influencing MC numbers in tissues. Note there are reports of SCF down-regulation of Kit in mature MC over several hours (49). However, the ability SCF to down-regulate Kit may not be meaningful in interpreting our experiments that take place over 60 min and involve use of progenitor MC. However, in the final anal-

ysis, Kit<sup>+</sup> progenitor cells did migrate, although one could argue that the method of analysis might underestimate the number of cells migrating, and the conclusion remains valid.

The possibility that tissue 5-HT levels, which increase in inflammation of the dermis, might play a role in MC recruitment (5–8) is an intriguing possibility, and may be relevant in inflammation of other tissues. For instance, 5-HT is known to be present in pulmonary tissues where the source may be pulmonary neuroendocrine cells (50, 51) or platelets (52), and thus could facilitate MC accumulation in inflammatory conditions of the lung. Similarly, 5-HT is known to be synthesized by enterochromaffin cells and nerve ganglia in the gastrointestinal system (53–55), and might influence accumulation of MC at these sites. In summary, we, for the first time, have shown that 5-HT has the ability to influence the biology of the MC. Data additionally clearly document that the effects of 5-HT are principally through the HT<sub>1A</sub> receptor. These observations also support the conclusion that pharmaceutical agents that more selectively block the action of 5-HT<sub>1A</sub> receptor could be explored as adjunct therapies in the treatment of allergic inflammation.

## Acknowledgments

We thank Dr. Michael Beaven for help with calcium determinations.

## Disclosures

The authors have no financial conflict of interest.

## References

- Galli, S. J., J. Wedemeyer, and M. Tsai. 2002. Analyzing the roles of mast cells and basophils in host defense and other biological responses. *Int. J. Hematol.* 75: 363–369.
- Maurer, M., T. Theoharides, R. D. Granstein, S. C. Bischoff, J. Bienenstock, B. Henz, P. Kovanen, A. M. Piliponsky, N. Kambe, H. Vliagoftis, et al. 2003. What is the physiological function of mast cells? *Exp. Dermatol.* 12: 886–910.
- Mekori, Y. A., and D. D. Metcalfe. 2000. Mast cells in innate immunity. *Immunol. Rev.* 173: 131–140.
- Metcalfe, D. D., D. Baram, and Y. A. Mekori. 1997. Mast cells. *Physiol. Rev.* 77: 1033–1079.
- Matsuda, H., H. Ushio, G. P. Geba, and P. W. Askenase. 1997. Human platelets can initiate T cell-dependent contact sensitivity through local serotonin release mediated by IgE antibodies. *J. Immunol.* 158: 2891–2897.
- Lundeberg, L., E. Sundstrom, K. Nordlind, A. Verhofstad, and O. Johansson. 1999. Serotonin in human allergic contact dermatitis. *Ann. NY Acad. Sci.* 885: 422–426.
- Lundeberg, L., H. El-Nour, S. Mohabbati, M. Morales, E. Azmitia, and K. Nordlind. 2002. Expression of serotonin receptors in allergic contact eczematous human skin. *Arch. Dermatol. Res.* 294: 393–398.
- Lundeberg, L., Y. Liang, E. Sundstrom, K. Nordlind, A. Verhofstad, S. Liden, and O. Johansson. 1999. Serotonin in human allergic contact dermatitis: an immunohistochemical and high-performance liquid chromatographic study. *Arch. Dermatol. Res.* 291: 269–274.
- Lechin, F., B. van der Dijks, B. Orozco, H. Jara, I. Rada, M. E. Lechin, and A. E. Lechin. 1998. Neuropharmacologic treatment of bronchial asthma with the antidepressant tianeptine: a double-blind, crossover placebo-controlled study. *Clin. Pharmacol. Ther.* 64: 223–232.
- Levy, L. H., E. Mendes, and A. U. Cintra. 1961. 5-Hydroxytryptamine in the bronchial sputum of asthmatic patients. *Acta Allergol.* 16: 121–127.
- Gresele, P., E. Ribaldi, S. Grasselli, T. Todisco, and G. G. Nenci. 1987. Evidence for platelet activation in allergic asthma. *Agents Actions. Suppl.* 21: 119–128.
- Pitchford, S. C., H. Yano, R. Lever, Y. Riffio-Vasquez, S. Ciferri, M. J. Rose, S. Giannini, S. Momi, D. Spina, B. O'Connor, et al. 2003. Platelets are essential for leukocyte recruitment in allergic inflammation. *J. Allergy Clin. Immunol.* 112: 109–118.
- Cazzola, I., and M. G. Matera. 2000. 5-HT modifiers as a potential treatment of asthma. *Trends Pharmacol. Sci.* 21: 13–16.
- Cazzola, M., G. Assogna, G. Lucchetti, G. Cicchitto, and G. D'Amato. 1990. Effect of ketanserin, a new blocking agent of the 5-HT<sub>2</sub> receptor, on airway responsiveness in asthma. *Allergy* 45: 151–153.
- Raymond, J. R., Y. V. Mukhin, A. Gelasco, J. Turner, G. Collinsworth, T. W. Gettys, J. S. Grewal, and M. N. Garnovskaya. 2001. Multiplicity of mechanisms of serotonin receptor signal transduction. *Pharmacol. Ther.* 92: 179–212.
- Betten, A., C. Dahlgren, S. Hermodsson, and K. Hellstrand. 2001. Serotonin protects NK cells against oxidatively induced functional inhibition and apoptosis. *J. Leukocyte Biol.* 70: 65–72.
- Freire-Garabal, M., M. J. Nunez, J. Balboa, P. Lopez-Delgado, R. Gallego, T. Garcia-Caballero, M. D. Fernandez-Roel, J. Brenlla, and M. Rey-Mendez. 2003. Serotonin upregulates the activity of phagocytosis through 5-HT<sub>1A</sub> receptors. *Br. J. Pharmacol.* 139: 457–463.

18. Idzko, M., E. Panther, C. Stratz, T. Muller, H. Bayer, G. Zissel, T. Durk, S. Sorichter, F. Di Virgilio, M. Geissler, et al. 2004. The serotonergic receptors of human dendritic cells: identification and coupling to cytokine release. *J. Immunol.* 172: 6011–6019.
19. Boehme, S. A., F. M. Lio, L. Sikora, T. S. Pandit, K. Lavrador, S. P. Rao, and P. Sriramarao. 2004. Cutting edge: serotonin is a chemotactic factor for eosinophils and functions additively with eotaxin. *J. Immunol.* 173: 3599–3603.
20. Bondesson, L., K. Nordlind, V. Mutt, and S. Liden. 1996. Inhibitory effect of vasoactive intestinal polypeptide and ketanserin on established allergic contact dermatitis in man. *Acta Derm. Venereol.* 76: 102–106.
21. Iken, K., S. Chheng, A. Fargin, A. C. Goulet, and E. Kouassi. 1995. Serotonin upregulates mitogen-stimulated B lymphocyte proliferation through 5-HT<sub>1A</sub> receptors. *Cell Immunol.* 163: 1–9.
22. Parks, C. L., P. S. Robinson, E. Sibille, T. Shenk, and M. Toth. 1998. Increased anxiety of mice lacking the serotonin<sub>1A</sub> receptor. *Proc. Natl. Acad. Sci. USA* 95: 10734–10739.
23. Tkaczyk, C., V. Horejsi, S. Iwaki, P. Draber, L. E. Samelson, A. B. Satterthwaite, D. H. Nahm, D. D. Metcalfe, and A. M. Gilfillan. 2004. NTAL phosphorylation is a pivotal link between the signaling cascades leading to human mast cell degranulation following Kit activation and FcεRI aggregation. *Blood* 104: 207–214.
24. Weller, C. L., S. J. Collington, J. K. Brown, H. R. Miller, A. Al-Kashi, P. Clark, P. J. Jose, A. Hartnell, and T. J. Williams. 2005. Leukotriene B<sub>4</sub>, an activation product of mast cells, is a chemoattractant for their progenitors. *J. Exp. Med.* 201: 1961–1971.
25. Tkaczyk, C., Y. Okayama, D. D. Metcalfe, and A. M. Gilfillan. 2004. Fcγ receptors on mast cells: activatory and inhibitory regulation of mediator release. *Int. Arch. Allergy Immunol.* 133: 305–315.
26. Kirshenbaum, A. S., J. P. Goff, T. Semere, B. Foster, L. M. Scott, and D. D. Metcalfe. 1999. Demonstration that human mast cells arise from a progenitor cell population that is CD34<sup>+</sup>, c-kit<sup>+</sup>, and expresses aminopeptidase N (CD13). *Blood* 94: 2333–2342.
27. Kulka, M., and D. D. Metcalfe. 2005. High-resolution tracking of cell division demonstrates differential effects of TH1 and TH2 cytokines on SCF-dependent human mast cell production in vitro: correlation with apoptosis and Kit expression. *Blood* 105: 592–599.
28. Kirshenbaum, A. S., and D. D. Metcalfe. 2005. Growth of human mast cells from bone marrow and peripheral blood-derived CD34<sup>+</sup> pluripotent progenitor cells. *Methods Mol. Biol.* 315: 105–112.
29. Chaves-Dias, C., T. R. Hundley, A. M. Gilfillan, A. S. Kirshenbaum, J. R. Cunha-Melo, D. D. Metcalfe, and M. A. Beaven. 2001. Induction of telomerase activity during development of human mast cells from peripheral blood CD34<sup>+</sup> cells: comparisons with tumor mast-cell lines. *J. Immunol.* 166: 6647–6656.
30. Kulka, M., L. Alexopoulou, R. A. Flavell, and D. D. Metcalfe. 2004. Activation of mast cells by double-stranded RNA: evidence for activation through Toll-like receptor 3. *J. Allergy Clin. Immunol.* 114: 174–182.
31. Okayama, Y., C. Tkaczyk, D. D. Metcalfe, and A. M. Gilfillan. 2003. Comparison of FcεRI- and FcγRI-mediated degranulation and TNF-α synthesis in human mast cells: selective utilization of phosphatidylinositol-3-kinase for FcγRI-induced degranulation. *Eur. J. Immunol.* 33: 1450–1459.
32. Liao, F., A. K. Shirakawa, J. F. Foley, R. L. Rabin, and J. M. Farber. 2002. Human B cells become highly responsive to macrophage-inflammatory protein-3α/CC chemokine ligand-20 after cellular activation without changes in CCR6 expression or ligand binding. *J. Immunol.* 168: 4871–4880.
33. Tkaczyk, C., D. D. Metcalfe, and A. M. Gilfillan. 2002. Determination of protein phosphorylation in FcεRI-activated human mast cells by immunoblot analysis requires protein extraction under denaturing conditions. *J. Immunol. Methods* 268: 239–243.
34. Tkaczyk, C., M. A. Beaven, S. M. Brachman, D. D. Metcalfe, and A. M. Gilfillan. 2003. The phospholipase Cγ1-dependent pathway of FcεRI-mediated mast cell activation is regulated independently of phosphatidylinositol 3-kinase. *J. Biol. Chem.* 278: 48474–48484.
35. Vliagoftis, H. 2002. Thrombin induces mast cell adhesion to fibronectin: evidence for involvement of protease-activated receptor-1. *J. Immunol.* 169: 4551–4558.
36. Ebersole, B. J., C. A. Diglio, D. W. Kaufman, and K. A. Berg. 1993. 5-Hydroxytryptamine<sub>1</sub>-like receptors linked to increases in intracellular calcium concentration and inhibition of cyclic AMP accumulation in cultured vascular smooth muscle cells derived from bovine basilar artery. *J. Pharmacol. Exp. Ther.* 266: 692–699.
37. Frevert, C. W., V. A. Wong, R. B. Goodman, R. Goodwin, and T. R. Martin. 1998. Rapid fluorescence-based measurement of neutrophil migration in vitro. *J. Immunol. Methods* 213: 41–52.
38. Dastyh, J., D. Taub, M. C. Hardison, and D. D. Metcalfe. 1998. Tyrosine kinase-deficient Wv c-kit induces mast cell adhesion and chemotaxis. *Am. J. Physiol.* 275: C1291–C1299.
39. Vega, E., and M. I. Rudolph. 2002. Characterization of oxytocin receptors and serotonin transporters in mast cells. *Endocrine* 18: 167–172.
40. Huang, J., G. Li, J. Xiang, D. Yin, and R. Chi. 2004. Immunohistochemical study of serotonin in lesions of psoriasis. *Int. J. Dermatol.* 43: 408–411.
41. Kirshenbaum, A. S., J. P. Goff, S. W. Kessler, J. M. Mican, K. M. Zsebo, and D. D. Metcalfe. 1992. Effect of IL-3 and stem cell factor on the appearance of human basophils and mast cells from CD34<sup>+</sup> pluripotent progenitor cells. *J. Immunol.* 148: 772–777.
42. Mekori, Y. A., C. K. Oh, and D. D. Metcalfe. 1993. IL-3-dependent murine mast cells undergo apoptosis on removal of IL-3: prevention of apoptosis by c-kit ligand. *J. Immunol.* 151: 3775–3784.
43. Wingren, U., A. Westeson, and L. Enerback. 1983. Storage and turnover of histamine, 5-hydroxytryptamine and heparin in rat peritoneal mast cells in vivo. *Int. Arch. Allergy Appl. Immunol.* 70: 193–199.
44. Kushnir-Sukhov, N. M., J. M. Brown, Y. Wu, A. Kirshenbaum, and D. D. Metcalfe. 2006. Human mast cells are capable of serotonin synthesis and release. *J. Allergy Clin. Immunol.* In press.
45. Kraft, S., T. Fleming, J. M. Billingsley, S. Y. Lin, M. H. Jouvin, P. Storz, and J. P. Kinet. 2005. Anti-CD63 antibodies suppress IgE-dependent allergic reactions in vitro and in vivo. *J. Exp. Med.* 201: 385–396.
46. Swindle, E. J., J. A. Hunt, and J. W. Coleman. 2002. A comparison of reactive oxygen species generation by rat peritoneal macrophages and mast cells using the highly sensitive real-time chemiluminescent probe pholasin: inhibition of antigen-induced mast cell degranulation by macrophage-derived hydrogen peroxide. *J. Immunol.* 169: 5866–5873.
47. Taylor, A. M., S. J. Galli, and J. W. Coleman. 1995. Stem-cell factor, the kit ligand, induces direct degranulation of rat peritoneal mast cells in vitro and in vivo: dependence of the in vitro effect on period of culture and comparisons of stem-cell factor with other mast cell-activating agents. *Immunology* 86: 427–433.
48. Aune, T. M., H. W. Golden, and K. M. McGrath. 1994. Inhibitors of serotonin synthesis and antagonists of serotonin 1A receptors inhibit T lymphocyte function in vitro and cell-mediated immunity in vivo. *J. Immunol.* 153: 489–498.
49. Baghestanian, M., H. Agis, D. Bevec, H. C. Bankl, R. Hofbauer, H. G. Kress, J. H. Butterfield, M. R. Muller, L. K. Ashman, W. Fureder, et al. 1996. Stem cell factor-induced downregulation of c-kit in human lung mast cells and HMC-1 mast cells. *Exp. Hematol.* 24: 1377–1386.
50. Pan, J., I. Copland, M. Post, H. Yeger, and E. Cutz. 2006. Mechanical stretch-induced serotonin release from pulmonary neuroendocrine cells: implications for lung development. *Am. J. Physiol.* 290: L185–L193.
51. Eddahibi, S., B. Raffestin, I. Pham, J. M. Launay, P. Aegerter, M. Sitbon, and S. Adnot. 1997. Treatment with 5-HT potentiates development of pulmonary hypertension in chronically hypoxic rats. *Am. J. Physiol.* 272: H1173–H1181.
52. Pitchford, S. C., Y. Riffo-Vasquez, A. Sousa, S. Momi, P. Gesele, D. Spina, and C. P. Page. 2004. Platelets are necessary for airway wall remodeling in a murine model of chronic allergic inflammation. *Blood* 103: 639–647.
53. Fujimiya, M., K. Okumiya, T. Yamane, and T. Maeda. 1997. Distribution of serotonin-immunoreactive nerve cells and fibers in the rat gastrointestinal tract. *Histochem. Cell Biol.* 107: 105–114.
54. Oshima, S., M. Fujimura, and M. Fukimiya. 1999. Changes in number of serotonin-containing cells and serotonin levels in the intestinal mucosa of rats with colitis induced by dextran sodium sulfate. *Histochem. Cell Biol.* 112: 257–263.
55. Gershon, M. D. 2003. Serotonin and its implication for the management of irritable bowel syndrome. *Rev. Gastroenterol. Disord.* 3(Suppl. 2): S25–S34.
56. Martel, F., R. Monteiro, and C. Lemos. 2003. Uptake of serotonin at the apical and basolateral membranes of human intestinal epithelial (Caco-2) cells occurs through the neuronal serotonin transporter (SERT). *J. Pharmacol. Exp. Ther.* 306: 355–362.