

IgE-Mediated Mast Cell Activation Induces Langerhans Cell Migration In Vivo¹

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Langerhans cells and mast cells are both resident in large numbers in the skin and act as sentinel cells in host defense. The ability of mast cells to induce Langerhans cell migration from the skin to the draining lymph node in vivo was examined. Genetically mast cell-deficient (W/Wv) mice and control mice were sensitized with IgE Ab in the ear pinna. Seven to 14 days later, mice were challenged with Ag i.v. After a further 18–24 h, epidermal sheets and draining auricular lymph nodes were examined using Langerin/CD207 immunostaining. In mast cell-containing mice, a significant decrease in the number of Langerhans cells was observed at epidermal sites of mast cell activation. A significant increase in total cellularity and accumulation of Langerin-positive dendritic cells was observed in the auricular lymph nodes, draining the sites of IgE-mediated mast cell activation. These changes were not observed in W/Wv mice, but were restored by local mast cell reconstitution. Treatment of mast cell-containing mice with the H2 receptor antagonist cimetidine significantly inhibited the observed IgE/Ag-induced changes in Langerhans cell location. In contrast, Langerhans cell migration in response to LPS challenge was not mast cell dependent. These data directly demonstrate the ability of mast cells to induce dendritic cell migration to lymph nodes following IgE-mediated activation in vivo by a histamine-dependent mechanism. *The Journal of Immunology*, 2004, 173: 5275–5282.

In atopic individuals, IgE Abs are detected to a large number of environmental Ags (1) which sensitize mast cells and basophils, for subsequent responses to allergen. Sensitization to environmental Ags is dependent on effective Ag presentation within the draining lymph nodes (LNs),³ a process in which the migration of resident tissue dendritic cells plays a key role.

Langerhans cells (LC) are immature dendritic cells found in the epidermal layer of the skin. They are characterized by the presence of Birbeck granules, expression of high levels of MHC class II, and a characteristic dendritic morphology. Recently, Langerin/CD207, a transmembrane C-type lectin and an unconventional endocytic receptor associated with the formation of the Birbeck granules, has been identified as a LC marker (2). LC act as sentinels for immune responses by capturing Ag in the epidermis in response to appropriate signals and by maturing and migrating into the draining LN where they become very potent in Ag presentation (3, 4). A number of cytokines are important inducers of LC maturation and/or migration, including GM-CSF (5), TNF, and IL-1 β (6, 7). Recombinant TNF and IL-1 β induce a significant migration of LC from the epidermis and a consequent accumulation of dendritic cells in the draining LN (8–10).

Mast cells are also present in the skin and are mostly studied in the context of allergy, but also have an important role in host defense. They express high-affinity Fc ϵ RI and IgE-mediated activation leads to the release of multiple preformed, newly formed, and lipid mediators. Preformed mediators, include histamine and granule-associated TNF, which induce dendritic cell maturation (11, 12). Mast cells produce a number of cytokines in the hours following IgE-mediated activation with known effects on dendritic cells including TNF, IL-1 β , and GM-CSF (13–15). CD40 ligand is also expressed by mast cells, and is important in regulating dendritic cell migration in vivo (16). Exosomes from IgE-activated human mast cells have recently been shown to induce human dendritic cell maturation in vitro (17). IL-1 β , TNF, and GM-CSF have all been clearly demonstrated to have the ability to induce dendritic cell maturation in vivo or in vitro (5, 6, 8, 9, 18). A study by Alard et al. (19) suggested that mast cell-derived TNF might inhibit contact sensitization through effects on dendritic cells in mice. A recent report has also demonstrated that mast cell-derived TNF is primarily responsible for LN swelling following bacterial or compound 48/80-induced mast cell activation (20), while histamine did not contribute significantly to this response. Dendritic cells have, however, been shown to express H1, H2, and H3 receptors and to undergo significant maturational changes as a result of histamine treatment (11, 21–23).

In this study, we examined the role of mast cells in LC migration from the skin to the draining auricular LN using a mouse model. We demonstrate that local IgE-mediated mast cell degranulation provides an effective signal for LC migration to the draining LN in vivo, in the absence of additional local Ag or TLR activator administration.

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Materials and Methods

Mice

C57BL/6 mice, as well as genetically mast cell-deficient WBB6F₁-W/Wv (W/Wv) mice and their normal congenic WBB6F₁^{+/+} (+/+) mice (The Jackson Laboratory, Bar Harbor, ME) were used. All mice were males, 6–10 wk of age. Mice were housed under conventional conditions with food and water provided ad libitum. All experiments were approved by the animal research ethics boards of Dalhousie University.

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³ Abbreviations used in this paper: LN, lymph node; LC, Langerhans cell; i.d., intradermal; TNF, trinitrophenyl.

Induction of local skin reactions

Mice received an intradermal (i.d.) injection into the ear pinna of 0.07 mg of purified mouse anti-trinitrophenyl (TNP) IgE prepared from the B cell line TIB-141 (American Type Culture Collection, Manassas, VA) in 25 μ l of PBS, mouse anti-dansyl IgE (BD Pharmingen, San Diego, CA) in 25 μ l of PBS, or diluent control (as stated). After a resting period, to allow excess IgE to dissipate (7–14 days), mice were challenged with an i.v. injection of 5 mg/ml TNP-BSA (Biosearch Technologies, Novato, CA) in 250 μ l of saline. Mice were sacrificed and their ear pinnae were removed after 18 h or draining auricular LN were excised after 24 h. For experiments using locally mast cell-reconstituted W/W^v mice both ear pinnae were injected with anti-TNP IgE 14 days before i.v. injection of TNP-BSA. In additional experiments, one ear pinna was injected with anti-TNP IgE and the other with anti-dansyl IgE 14 days before i.v. TNP administration. Some animals were also examined 14 days after i.d. IgE injection, but in the absence of i.v. TNP-BSA administration.

Examining the role of histamine receptors

To determine the role of histamine H1 and H2 receptors, 14 days following IgE or control injection in the ear pinnae mice were treated with either the H1 antagonist pyrilamine, 50 mg/kg i.p., or the H2 antagonist cimetidine, 50 mg/kg i.p. (Sigma-Aldrich, St. Louis, MO). The doses of histamine receptor antagonists used were based on previous reports of a single dose in vivo used in mice (24–26). Ninety minutes later the animals were injected i.v. with TNP-BSA and responses were evaluated as described below following harvest of LN cells 24 h later.

Examining the role of serotonin

To determine the role of serotonin (5-hydroxytryptamine), 14 days following IgE or control injection in the ear pinnae mice were injected i.p. with 1.6 mg/kg ketanserin, a 5-hydroxytryptamine receptor antagonist (Research Biochemical International, Oakville, Ontario, Canada). Ninety minutes later the animals were injected i.v. with TNP-BSA and responses were evaluated as described below following harvest of LN cells 24 h later.

Induction of response to LPS

To examine the effect of LPS, mice were injected with 0.25 μ g of *Escherichia coli* LPS serotype 055:B5 (Sigma-Aldrich) in 25 μ l of saline in the ear pinna or with diluent control. Draining auricular nodes were examined after 18 h.

Preparation and analysis of epidermal sheets

Epidermal sheets were prepared as previously described by Baker et al. (27). Briefly, mice were sacrificed and their ears were split into dorsal and ventral halves by forceps. The dorsal halves were incubated for 2 h at 37°C with 10 mM disodium ethylenediamine tetraacetic acid (BDH, Toronto, Canada) dissolved in PBS. Ears were washed with PBS and then under a dissecting microscope the epidermis was peeled off using forceps. The epidermis was then fixed in cold acetone (–20°C) for 2 min, washed in PBS, and stored at 4°C until immunohistochemical staining.

Preparation of auricular LN cells

After the mice were challenged with TNP-BSA, the draining (auricular) LNs were excised and, in some experiments (as stated), pooled for each animal. A LN-derived single-cell suspension was prepared, washed, and resuspended in RPMI 1640 medium. Viable cell counts were performed using trypan blue, and the cell concentration was adjusted to 1×10^6 cells/ml. Cytochrome preparations of the LN-derived single-cell suspension were made and cells were stained with anti-Langerin/CD207 or control Ab according to the immunohistochemical procedure described below.

Immunohistochemistry and cell counts

Immunostaining was performed on epidermal skin sheets, LN frozen sections, and cytochrome preparation of LN cell suspensions. Following fixation, sheets or slides were washed with PBS and incubated with 3% BSA (Boehringer Mannheim, Mannheim, Germany) for 1 h. Sheets or slides were then incubated with either anti-Langerin Ab/CD207, clone 959F3 rat IgG1 (DCGM4) (0.75 μ g/ml) (courtesy of Dr. S. Saeland, Schering-Plough, Kenilworth, NJ) or in some experiments (as stated) with an anti-mouse MHC class II Ab MRC OX-3 (12.5 μ g/ml; Cedarlane Laboratories, Hornby, Ontario, Canada) or an isotype-matched control Ab for 16–18 h at room temperature. Sheets or slides were washed and incubated with biotinylated goat anti-mouse Ab, then detected using streptavidin-HRP (DAKO LSAB-2 System; DakoCytomation, Mississauga, Ontario,

Canada) and aminoethylcarbazole substrate (Zymed Laboratories, San Francisco, CA). A randomly chosen 10 fields (0.25 mm²) of epidermal sheets were counted and at least 2000 cells were assessed per slide for examination of cell suspensions. All counts were performed on coded slides with the observer being unaware which slides belonged to which experimental group.

Mast cell reconstitution

Bone marrow-derived mast cells were generated from the bone marrow of WBB6F₁^{+/+} (+/+) mice according to the method of Tertian et al. (28), a minimum of 98% pure cells was used. W/W^v mice were locally reconstituted as previously described (29). Cells derived from +/+ mice (0.5×10^6) were locally injected into the right ear pinna of W/W^v mice and allowed to mature for 10 wk.

Evaluation of anti-TNP Ab responses

Serum Ab responses against TNP were evaluated by ELISA. Briefly, the wells of ELISA plates were coated with TNP-keyhole limpet hemocyanin (Calbiochem, San Diego, CA) and nonspecific binding was blocked by incubation with 1% BSA. A series of dilutions of serum samples from immunized and nonimmunized mice were added to the washed plate and incubated for 16–18 h. Binding of Abs was detected using a biotinylated goat anti-mouse IgG/IgA/IgM Ab (Cedarlane Laboratories) followed by a commercial amplification substrate system (Invitrogen Life Technologies, Grand Island, NY).

Flow cytometry

Cells were washed twice with 2% FBS/PBS, fixed with 1% paraformaldehyde/PBS for 30 min, and washed with 2% FBS/PBS. The cells were permeabilized with 0.1% saponin/2% FBS/PBS for 20 min and stained with anti-Langerin (CD207) Ab, anti-fascin Ab, or an isotype control for 20 min. Following staining the cells were washed twice by gentle shaking in 0.1% saponin/2% FBS/PBS for 5 min before centrifugation. Cells were incubated with the secondary Ab, an FITC-labeled mouse anti-rat IgG Ab, and a PE-labeled goat anti-mouse IgG Ab for 20 min, followed by the same washing procedure. To block free Ab binding sites, the cells were incubated with 5% rat serum and 20 μ g/ml hamster IgG. Cells were washed twice with 0.1% saponin/2% FBS/PBS, once with 2% FBS/PBS, and then stained for cell surface markers with a PE-conjugated anti-CD86, CD40, or isotype control as well as an allophycocyanin-conjugated CD11c or isotype control. Finally, the cells were washed twice with 2% FBS/PBS. For flow cytometric analysis, 3×10^4 cells were acquired with a FACSCalibur (BD Biosciences, Mountain View, CA) and analyzed with CellQuest (BD Biosciences). All Abs were purchased from (BD Biosciences) except for anti-fascin, which was purchased from DakoCytomation and the PE-labeled goat anti-mouse IgG Ab which was purchased from Molecular Probes (Leiden, The Netherlands).

Statistical methods

Following confirmation of appropriate data distribution, differences between left and right epidermal sheets or left and right LNs were evaluated using a paired Student's *t* test. Differences between groups of animals were assessed using an unpaired Student's *t* test.

Results

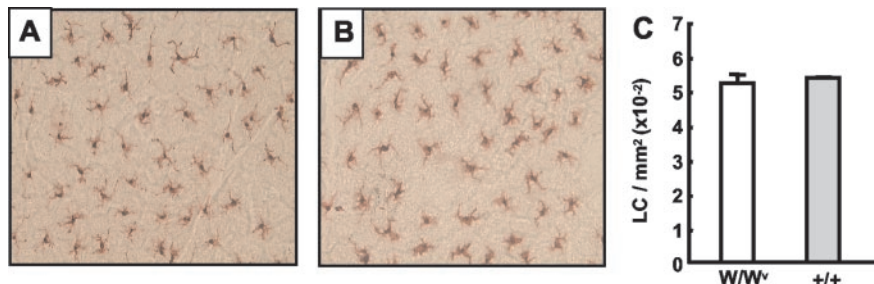
Mast cell-deficient mice exhibit a normal distribution of LC in the epidermis

The consequences of functional *c-kit* deficiency on the normal distribution of LC in the skin of W/W^v mice were examined to confirm the appropriateness of this mouse model for these studies. The number of Langerin/CD207-positive cells in the epidermis of untreated mast cell-deficient W/W^v mice was compared with those in the wild-type control +/+ mice. No significant differences in LC distribution or morphology were observed (Fig. 1).

IgE-mediated mast cell activation induces a reduction of LC in the skin and an increase in Langerin/CD207-positive dendritic cells in the draining LNs

IgE-mediated mast cell activation was used to selectively activate mast cells in one ear pinna. C57BL/6 mice were injected with anti-TNP IgE Ab into the right ear pinna and saline into the left ear

FIGURE 1. Mast cell-deficient *W/W^v* mice exhibit a normal distribution of LC in the epidermis. *A* and *B*, Immunohistochemical staining of LC using anti-Langerin/CD207 in ear pinna epidermal sheets obtained from mast cell-deficient *W/W^v* mice (*A*) compared with their wild-type control *+/+* mice, respectively. *C*, Mean $\pm$ SEM numbers of LC ($n = 4$). Note similar numbers of LC and similar distribution in *W/W^v* compared with wild-type control *+/+* mice.



pinna 14 days before Ag challenge with an i.v. injection of TNP-BSA. After another 18 h, selected on the basis of pilot experiments, the number of LC in the epidermal sheets was assessed. A significant ($p < 0.05$) reduction in the number of Langerin/CD207-positive LC was consistently observed in the right (IgE-injected) ears of the mice compared with their left (control) ears (Fig. 2, *A* and *B*). These results were confirmed using MHC class II as a second marker of LC (data not shown). The mean reduction in LC in the IgE-injected skin compared with the control skin was 16.4% (Fig. 2C) using Langerin/CD207 staining and 16.7% using MHC class II staining.

In separate animals, the auricular LNs draining ear pinnae that had been previously sensitized with anti-TNP IgE (as above) or with saline as control were excised 24 h after i.v. Ag challenge, and the total cellularity and number of Langerin-positive dendritic cells was determined. A 2.1-fold increase ($p < 0.02$) was observed in the total cell numbers recovered from nodes draining sites of IgE-mediated mast cell activation compared with control sites (Fig. 2D).

The decrease in LC at skin sites of IgE-mediated activation was mirrored by an increase in the number of Langerin-positive dendritic cells within the draining LNs (Fig. 2, *E* and *F*). A 2.3-fold increase ($p < 0.05$) was observed in the number of Langerin/CD207-positive cells/LN draining sites of IgE-mediated activation compared with control sites (Fig. 2, *G* and *H*). The specificity of Langerin/CD207 staining was confirmed using isotype control Ab in place of anti-Langerin/CD207; no positively stained dendritic cells were observed under these conditions (data not shown).

LC migration in *W/W^v* mast cell-deficient mice

To determine the role of mast cells in IgE-mediated LC migration, both mast cell-deficient *W/W^v* mice and their wild-type control *+/+* mice were injected with anti-TNP IgE Ab and challenged with i.v. Ag as described above. No significant decrease in the numbers of Langerin-positive cells was observed in the IgE-injected ear pinnae of *W/W^v* mice (Fig. 3A). However, a small but consistent increase in the number of Langerin/CD207-positive LC

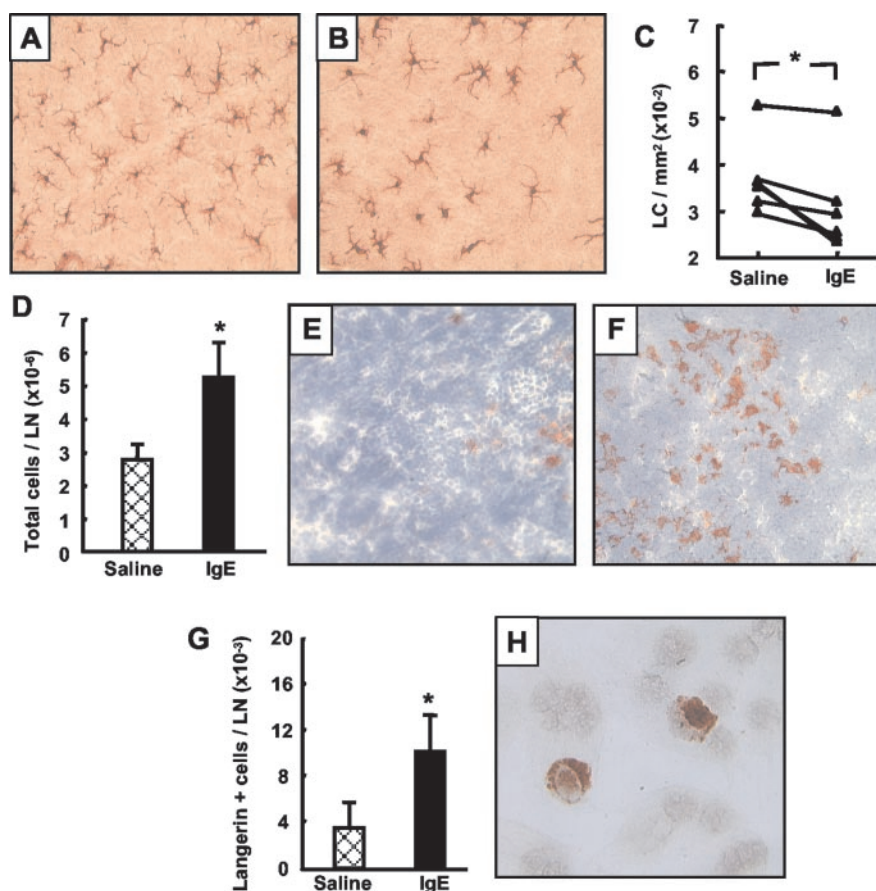


FIGURE 2. LC numbers decrease in the epidermis and increase in the draining LNs following IgE-mediated mast cell activation. Saline or anti-TNP IgE was given i.d. in ear pinnae 1 wk before the i.v. TNP-BSA injection, and tissues were harvested after 18 h for examination of ear tissue or 24 h for LNs. All tissues were stained using anti-Langerin/CD207 Ab. *A*, LC in epidermal sheet of saline-injected mouse ear pinna. *B*, Anti-TNP IgE-injected mouse ear pinna. *C*, LC density in epidermal sheets from individual mice ($n = 6$). *D*, Mean $\pm$ SEM number of total cells per draining LN. *E* and *F*, Frozen section of draining LNs from the nodes draining the saline-injected side and anti-TNP IgE-injected side, respectively. *G*, Mean $\pm$ SEM numbers of Langerin/CD207-positive dendritic cells per draining LN. *H*, Cytochrome preparation of draining LN cell suspension showing Langerin/CD207-positive dendritic cells. *, $p < 0.05$.

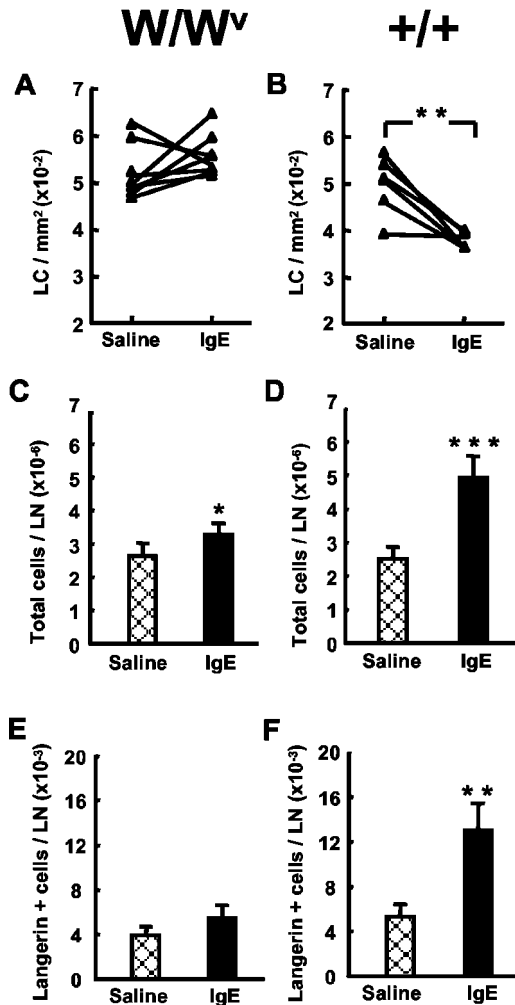


FIGURE 3. Mast cells are required for changes in LC distribution following local IgE-mediated activation. Genetically mast cell-deficient *W/Wv* mice and normal congenic *+/+* (mast cell-containing) mice received a saline injection as a control in one ear pinna and an anti-TNP IgE injection in the contralateral ear pinna. Seven days later, mice were injected with TNP-BSA i.v. and the numbers of their LC were examined after 18 h using anti-Langerin/CD207 immunohistochemistry. The density of LC stained with anti-Langerin in epidermal sheets from both *W/Wv* mice (A; $n = 8$), and from *+/+* mice (B; $n = 6$) was determined. Cells from the draining LNs were also examined 24 h after the i.v. challenge. Numbers of total cells per draining LN from *W/Wv* mice (C; $n = 10$) and *+/+* mice (D; $n = 10$). Numbers of Langerin/CD207-positive dendritic cells per draining LN from *W/Wv* mice (E; $n = 10$) and from *+/+* mice (F; $n = 10$). All numbers represent the mean values $\pm$ S.E.M. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

cells was observed. In control *+/+* mice, a 23% decrease in Langerin/CD207-positive LC cells ($p < 0.01$) was observed in the IgE-injected ear pinna compared with the control site (Fig. 3B). Consistent with these data, only a 25% increase ($p < 0.05$) in total LN cellularity was observed in *W/Wv* mice in the LNs draining the IgE-activated ear pinnae compared with controls (Fig. 3C). In contrast, a 2-fold increase ($p < 0.0001$) in total cellularity was observed in *+/+* mice in the LNs draining the IgE-activated side compared with control (Fig. 3D). No significant increase was observed in the numbers of Langerin/CD207-positive cells in the LNs of *W/Wv* mice (Fig. 3E). However, a 2.5-fold increase ($p < 0.01$) in the numbers of Langerin/CD207-positive cells was observed in control *+/+* mice (Fig. 3F). These observations suggested that the induction of LC migration following local IgE-mediated activation is mast cell dependent.

The anti-TNP Ab (IgG and IgM) response in *W/Wv* and control mice was determined 2 wk after i.v. Ag injection in a separate set of animals ($n = 5$ /group). There was no significant difference in the anti-TNP titer between the mast cell-deficient and mast cell-containing mice (data not shown). These observations confirm the ability of mast cell-deficient mice to mount a normal immune response following systemic Ag administration.

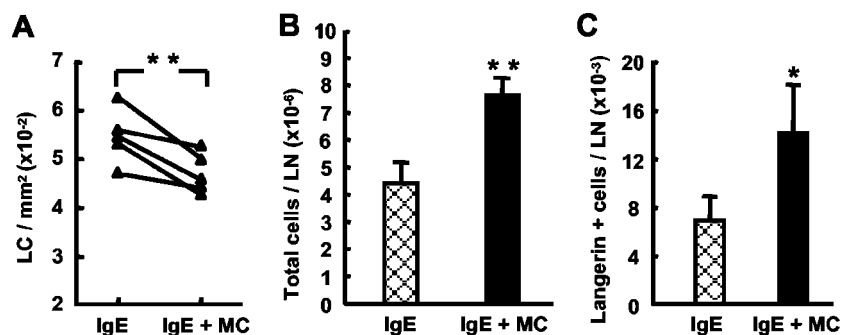
LC cells observed in draining LNs have undergone maturation

To examine the maturation status of LC within LNs draining sites of IgE-mediated mast cell activation, a flow cytometric approach was used. The dual Langerin-positive and CD11c-positive cells were examined for their expression of the well-defined maturation markers CD86, fascin, and CD40. LC at these sites were found to be on average ($n = 6$) 85% CD86 positive, 87% fascin positive, and 45% CD40 positive (data not shown). This marker profile is in keeping with a matured LC phenotype. The marker profile of LC was not significantly different between nodes draining sites of IgE-mediated mast cell activation and control sites.

LC migration in *W/Wv* mice with local mast cell reconstitution

To more directly address the role of the mast cell, *W/Wv* mice were locally reconstituted by i.d. injection with bone marrow-derived mast cells in the right ear only. After a 10-wk resting period, to allow mast cells to mature in vivo, mice were injected with anti-TNP IgE Ab in both ear pinnae. One week later, mice were challenged with TNP-BSA Ag i.v. and both epidermal sheets (18 h) and auricular nodes (24 h) were examined for their numbers of Langerin/CD207-positive dendritic cells. Examination of the epidermal sheets from the ear pinnae revealed that Langerin/CD207-positive cell numbers were significantly ($p < 0.01$) decreased in the mast cell-reconstituted site compared with the nonreconstituted site (Fig. 4A). A significant ($p < 0.01$) increase in total cellularity was observed in the LNs draining the mast cell-reconstituted ear

FIGURE 4. Local mast cell reconstitution restores LC migration. Mast cell-deficient *W/Wv* mice were injected with cultured bone marrow-derived mast cells in the right ear only. After 10 wk, both ears were injected with anti-TNP IgE and, after a further 7 days, the mice were challenged with TNP-BSA i.v. After 18 h, the number of LC in the ear pinna epidermis for individual mice ($n = 5$) was determined (A). After 24 h, cells from the draining auricular LNs ($n = 5$) were examined. B, Numbers of total cells per draining LN. C, Numbers of Langerin/CD207-positive dendritic cells per draining LN. All numbers represent the mean values $\pm$ SEM. *, $p < 0.05$; **, $p < 0.01$.



pinna (Fig. 4B). A significant ($p < 0.02$) increase in the number of Langerin/CD207-positive dendritic cells/LNs was also observed at this site (Fig. 4C).

LPS induces LC migration in mast cell-deficient W/Wv mice

LPS is a known inducer of dendritic cell migration (7) via interactions with TLRs, but does not induce mast cell degranulation (30). Both W/Wv mice and their wild-type control $+/+$ mice were injected with LPS into their right ear and saline into the left. After 18 h, mice were sacrificed and LNs were examined. In the draining LNs, there was a similar significant ($p < 0.01$) increase in the number of total cells in the LPS draining node compared with the control in both W/Wv mice (2-fold; Fig. 5A) and control $+/+$ mice (2.6-fold; Fig. 5B). In addition, the numbers of Langerin/CD207-positive dendritic cells showed a significant ($p < 0.05$ and $p < 0.01$) increase in the sites draining LPS-injected skin compared with control in both W/Wv mice (3.4-fold; Fig. 5C) and $+/+$ mice (4-fold; Fig. 5D).

Changes in LC numbers are dependent upon specific Ag administration

To investigate the mechanism of these responses in more detail, we focused on the changes in Langerin-positive cells and total cellularity observed in the auricular LNs draining sites of IgE-mediated mast cell activation. Murine LC do not express FcεRI (31); however, they do express the low-affinity FcεRII/CD23. There was no significant difference in the number of Langerin/CD207-positive cells found within the LNs draining IgE-injected or control (saline-injected) ear pinnae (Fig. 6, A and B). As further confirmation that the substantial dendritic cell responses observed in the LNs were

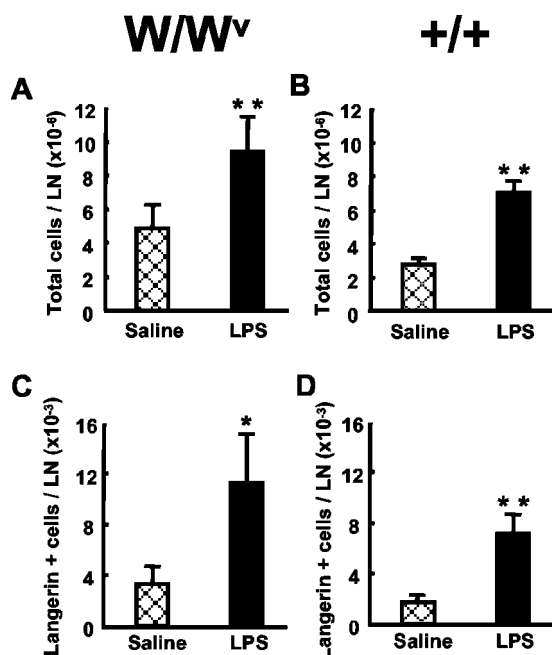


FIGURE 5. LPS induces similar levels of LC migration in W/Wv and control mice. Genetically mast cell-deficient W/Wv mice ($n = 5$) and their normal congenic $+/+$ mice ($n = 5$) received saline as a control in the left ear pinna and LPS ($0.75 \mu\text{g}$) in the right ear pinna. Eighteen hours later, mice were sacrificed and the total number of cells and Langerin/CD207-positive dendritic cells in the draining auricular LNs were examined. A and B, Mean $\pm$ SEM numbers of total cells per draining LNs from W/Wv mice and from $+/+$ mice, respectively. C and D, Mean $\pm$ SEM numbers of Langerin-positive dendritic cells per draining LNs from W/Wv mice and from $+/+$ mice, respectively. *, $p < 0.05$; **, $p < 0.01$.

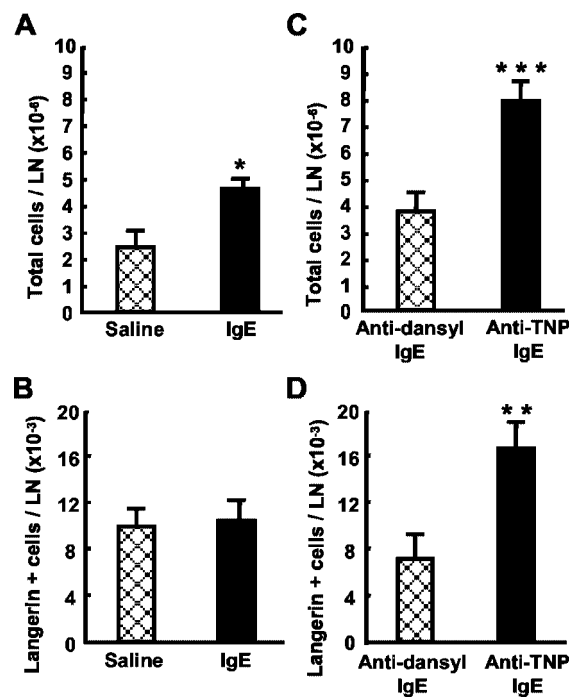


FIGURE 6. Changes in LC numbers are Ag and specific IgE dependent. A and B, Saline or anti-TNP IgE was given i.d. in the ear pinnae alone without further Ag challenge. LN tissues were harvested at day 14 and total cells and Langerin/CD207-positive cells were counted. A, Numbers of total cells per draining LN. B, Numbers of Langerin/CD207-positive dendritic cells per draining LN. C and D, Anti-dansyl IgE or anti-TNP IgE was given i.d. in the ear pinnae 14 days before the i.v. TNP-BSA challenge injection. LN tissues were harvested and total cells and Langerin/CD207-positive cells were counted. C, Numbers of total cells per draining LN. D, Numbers of Langerin/CD207-positive dendritic cells per draining LN. All values represent groups of six to eight animals, mean values $\pm$ SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

the result of Ag-specific IgE-mediated mast cell activation, one ear pinna of a group of six C57BL/6 mice was injected i.d. with anti-dansyl IgE while the other was injected with anti-TNP IgE. Fourteen days later, the mice were injected i.v. with TNP-BSA and after 24 h the draining auricular LNs were excised and examined for the number of Langerin/CD207-positive cells. Again, the LNs draining the site of IgE-mediated mast cell activation (injected with anti-TNP IgE) showed significantly increased numbers of Langerin/CD207-positive cells compared with the site that received anti-dansyl IgE injection alone (Fig. 6, C and D).

Consequences of H1 and H2 histamine receptor blockade

In view of the known effects of histamine on dendritic cell maturation and function, the consequences of pharmacologic blockade of either H1 receptors using pyrilamine or H2 receptors using cimetidine were examined. LNs draining sites of IgE-mediated mast cell activation were examined in C57BL/6 mice that had been pretreated with cimetidine, pyrilamine, or diluent (control) injection i.p. 90 min before i.v. Ag challenge of IgE-sensitized mice (anti-TNP IgE 14 days before Ag injection). The numbers of Langerin/CD207-positive cells migrating to the LN draining sites of IgE-mediated activation were similar in diluent-treated animals and following H1 blockade (Fig. 7, B and D). In marked contrast, following H2 blockade, increases in Langerin/CD207-positive cells in the auricular node draining sites of IgE-mediated activation were almost completely blocked (Fig. 7F; 90.5% inhibition compared with diluent-treated control group). The increase in total LN

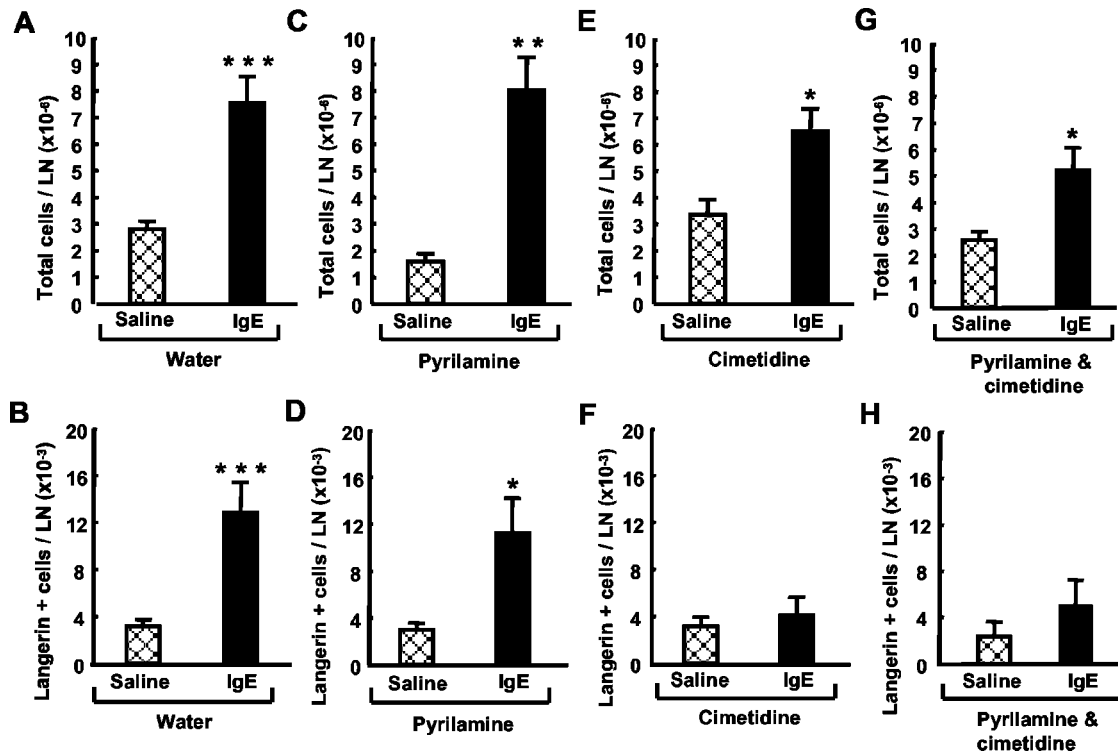


FIGURE 7. Histamine effect through H2 receptor is required for LC migration. Saline or anti-TNP IgE was injected i.d. in the ear pinnae. After 7 days, mice were injected i.p. with water (diluent control), pyrilamine (H1 antagonist), cimetidine (H2 antagonist), or a combination of cimetidine and pyrilamine 90 min before i.v. TNP-BSA Ag challenge injection. LN tissues were harvested and total cells and Langerin/CD207-positive cells were counted. A, Numbers of total cells per draining LN in diluent control-treated animals. B, Numbers of Langerin-positive dendritic cells per draining LN of diluent control-treated animals. C, Numbers of total cells per draining LN of pyrilamine-treated animals. D, Numbers of Langerin/CD207-positive dendritic cells per draining LN of pyrilamine-treated animals. E, Numbers of total cells per draining LN of cimetidine-treated animals. F, Numbers of Langerin/CD207-positive dendritic cells per draining LN of cimetidine-injected animals. G, Numbers of total cells per draining LN of cimetidine- and pyrilamine-treated animals. H, Numbers of Langerin/CD207-positive dendritic cells per draining LN of cimetidine- and pyrilamine-treated animals. All values represent groups of 6–12 animals, mean values $\pm$ SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

cellularity, as a result of IgE-mediated mast cell activation, was not significantly inhibited by cimetidine pretreatment of the animals. The effects of combined cimetidine and pyrilamine treatment were similar to those of cimetidine alone (Fig. 7, G and H).

To further evaluate the ability of histamine alone to mimic the effects of local mast cell degranulation of LC, separate groups of C57BL/6 mice were injected i.d. with 2.75 or 27.5 μ g of histamine. Twenty-four hours later the presence of LC in the draining LNs was evaluated. No significant LC increase in the draining LNs was observed following such histamine injection compared with diluent-injected controls (data not shown).

Serotonin is also found in large amounts in rodent mast cells. To examine the ability of this mediator to participate in the observed LC response, the serotonin receptor antagonist ketanserin was used. Ketanserin was completely ineffective in blocking LC migration in response to mast cell activation when used at in vivo doses that have been shown to be highly effective in blocking other serotonin-dependent events (32). Ketanserin was injected 90 min before Ag challenge, and both the number of total cells and Langerin-positive cells were determined. Total cells in the IgE-injected side were 5.6×10^6 cells vs 3.2×10^6 cells in the control side. Langerin-positive cells were 17×10^3 cells in the IgE-injected side vs 8.3×10^3 cells in the control-injected side with a $p < 0.0001$.

Discussion

This study directly demonstrates the role of mast cells in vivo in the regulation of LC migration. Both decreases in LC in the epi-

dermis following IgE/Ag challenge and increases in Langerin/CD207-positive cells in the draining auricular LNs were mast cell dependent using the W/W^v mast cell-deficient mouse model. Results of experiments using the H2 receptor blocker cimetidine strongly implicate histamine as the major mast cell mediator involved via interaction with the H2 receptors known to be present on dendritic cells.

Several different approaches confirmed that changes in LC populations were the result of mast cell activation and not IgE administration alone or IgE-Ag interactions on other cell types. First, when W/W^v mice were selectively reconstituted in one ear pinna with mast cells and then injected in both ear pinnae with IgE, before i.v. Ag challenge, a significant ($p < 0.01$) difference was observed between the mast cell-containing site and the nonreconstituted site. Both a decrease in Langerin/CD207-positive cells in the epidermal sheets where mast cells were present and an increase in Langerin/CD207-positive cells in the draining auricular LNs were observed when compared with the mast cell-deficient ear sites within the same animals (Fig. 4). Second, we confirmed that IgE injection into the ear pinna alone, in the absence of i.v. Ag challenge, did not induce changes in the number of Langerin/CD207-positive cells within the draining LNs when compared with saline-injected control ears in the same animals (Fig. 6B). As a further control for potential effects of IgE injection alone on LC migration, some animals were injected in both ears with IgEs of differing specificity. When Ag was injected i.v., only the LC populations from sites injected with IgE of appropriate specificity

were observed to substantially migrate from the ear epidermis to the draining node (Fig. 6D).

It is notable that the observed changes in Langerin/CD207-positive cells within the epidermal sheets were lower than might have been expected given the substantial increases in Langerin/CD207-positive cells within the draining auricular node. The scale of change in both the epidermal site and the draining node are consistent with that observed by others using direct injection of TNF and/or IL-1 β into the skin (9, 10). The small overall reduction in LC numbers may, in part, be explained by recruitment of new LC to the site of mast cell activation (33). Henri et al. (34) have demonstrated that the numbers of Langerin-positive cells in LNs draining the skin are normally regulated by a steady state of migration, consistent with the presence of some Langerin/CD207-positive cells in the control LNs. In addition, Stoitzner et al. (35) have shown that Langerin/CD207-positive cells in the skin-draining LNs are mostly mature. Changes in LC numbers in both the ear epidermis and draining LNs were consistent with IgE-mediated mast cell activation inducing LC migration in the context of a background level of continuous recruitment of immature LC to the skin and subsequent migration of cells to the draining LNs. The appropriateness of the specific auricular node selected for our study was confirmed by pilot experiments in which India ink was injected i.d. into the ear pinnae of mice (data not shown). This enabled us to isolate a single LN for study, reducing any contribution from other tissues to the changes in LC populations we examined. However, we cannot completely rule out the possibility that Langerin/CD207-positive cells from neighboring sites might contribute to the increase in Langerin/CD207-positive cells within the node.

The LC that migrated to draining LNs following IgE-mediated mast cell activation appear to have a similar phenotype to those found normally at this site and to have undergone some degree of maturation. A high percentage of Langerin/CD207-positive cells in these LNs were fascin and CD86 positive, a profile typical of a mature dendritic cell. It has been suggested that as LC mature in the LNs they will lose their expression of Langerin/CD207 so that both the numbers of LC migrating to the draining nodes and the degree of maturity, we are reporting, might be underestimates.

The ability of LPS administration to induce LC migration in both *W/W^v* and control *+/+* mice, to a similar extent, illustrates that mast cell activation is not required for LC migration in response to all stimuli. Although murine mast cells express the TLR4 receptor and have been shown to respond functionally by producing multiple cytokines in response to LPS (36–39), the mast cell contribution to LC mobilization, under our experimental conditions, was not significant. These data also illustrate that the LC in *W/W^v* mice are capable of being mobilized normally following appropriate stimulation, further confirming that the functional *c-kit* deficiency in *W/W^v* mice has not substantially altered the ability of LC to migrate normally. It is also notable that although changes in auricular LN cellularity in response to IgE/Ag-mediated activation were highly mast cell dependent, LPS injection induced similar changes in LN cellularity in both mast cell-containing and mast cell-deficient mice. These data suggest that mast cell-independent mechanisms for LN activation are mobilized following LPS injection in addition to the mast cell-derived TNF-dependent mechanisms that have been recently described (20).

The mechanism by which mast cells induce LC migration appears to be highly dependent upon histamine and H2 receptors. Our data demonstrate a critical role for H2 receptors in the migration of LC to draining LNs following IgE-mediated mast cell activation. It is notable, however, that H2 blockade was not effective as an inhibitor of mast cell-dependent increases in total LN cellu-

larity. These data are in keeping with recent studies suggesting that this process is mainly TNF dependent (20). H1 blockade did not significantly alter total LN cellularity or LC migration to draining LNs at sites of IgE-mediated activation. However, there was a slight trend toward reduced LC numbers in the activated LNs, which may reflect some cross-inhibition of H2 receptors by the relatively high doses of pyrilamine used in this study. Histamine up-regulates the expression of CD86, CD40, CD54, CD80, and MHC class II, as well as induces proinflammatory cytokine and chemokine production by human immature dendritic cells through H1 and H2 receptors (11). However, histamine alone does not up-regulate CCR7, which is thought to be crucial for dendritic cell migration and can be regulated by IL-1 β or TNF (40, 41). Although histamine is clearly required, it is probably not the only necessary mast cell-derived factor involved in the process of mast cell-dependent LC mobilization. Indeed, administration of histamine alone into ear skin did not induce LC migration, suggesting a role for other factors induced from mast cells or neighboring cells as a result of IgE-mediated mast cell activation. One potentially important factor is TNF, which is found both preformed in mast cells and newly synthesized following activation (14). Intradermal administration of TNF induces LC migration and dendritic cell accumulation in the draining LNs in mice (8, 9). Other additional candidate inducers of mast cell-mediated LC migration include IL-1 β , GM-CSF, and CD40 ligand, which have been implicated in dendritic cell migration or maturation and are expressed by activated human and rodent mast cells (42, 43). Mast cell exosomes may provide many of these mediators, or additional signals and have been shown to up-regulate dendritic cell MHC class II, CD80, CD86, and CD40 molecules. Mast cell exosomes, containing histamine, also induce dendritic cells to become efficient APCs, eliciting both IgG1 and IgG2a Abs in mice following immunization with exosome-associated Ags (17).

Complement activation has also been implicated as an important signal for mast cell activation and subsequent interaction with dendritic cells. Tsuji et al. (44) have shown that C5 is required early for the elicitation phase of contact sensitivity and delayed-type hypersensitivity. C5 was required for macrophage chemotactic activity and the late production of IFN- γ . C5a has also been shown to induce serotonin release from mast cells which induces endothelial cells activation and Th1 cells extravasation (45). The design of the current studies largely preclude an important role for complement locally within one ear as compared with the contralateral ear. Mouse IgE, particularly when bound to Fc ϵ RI, is not considered an effective complement activator. Free IgE has a relatively short (12-h) half-life in rodents and is injected 7–14 days before i.v. Ag administration in our experiments.

The data reported here do not directly implicate mast cells in the process of Ag presentation or contact sensitization, which has previously been implied (46, 47). For example, Bryce et al. (48) have shown that mast cells are required for contact sensitivity and dendritic cell reduction in the epidermis following hapten exposure. However, our results indicate that LC migration to draining LNs can be induced by IgE-mediated mast cell activation. Further signals may then be required to allow such LC to present Ag effectively. The process of mast cell-induced LC migration suggests a potential role in the perpetuation of chronic allergic disease. Once an IgE response to a specific environmental Ag has been established, subsequent exposure may lead to mast cell activation and release of mediators. Potentially, these mediators may enhance the migration of further local LC, carrying further environmental Ags to the draining LNs. This process could increase the possibility that additional IgE responses would occur in response to common environmental Ags. Histamine has been demonstrated to enhance

Th2 responses through effects on dendritic cells (21). Our current studies demonstrate that mast cell-derived signals, including histamine, also provide key stimuli for inducing LC migration in vivo. The importance of mast cell-dependent mobilization of LC to both allergic sensitization and the rapid development of effective immunity to pathogens will be important topics for future studies.

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