cubated with FITC-labeled avidin (1:500) and an Alexa546-conjugated anti-rat IgG antibody (1:1000) for 1 h. The fluorescent images were obtained using a fluorescence microscope Nikon Eclipse TE300 (Nikon, Tokyo, Japan) and analyzed using the IPLab 3.5.5/J software. The differential interference contrast microscopy (DIC) images were obtained using the same system. The confocal images were obtained with a confocal laser scanning microscope (LSM5 Pascal; Carl Zeiss, Oberkochen, Germany) with C-Apochromat ×63/1.2 and analyzed using the LSM5 Pascal software.

Measurement of HA

The concentrations of HA in the medium were determined using the ELISA-like system for HA with bHABP, according to the manufacturer's instruction.

Measurement of Histamine

Mast cells were lysed in PBS containing 2 M NaCl and 0.5% Triton X-100 at 4 °C. The lysate of mast cells and the ear tissues were centrifuged at 10 000 *g* for 30 min at 4 °C. The resultant supernatant was subjected to the assays for histamine as described earlier.[13]

Cell Proliferation Assay

Cell proliferation was evaluated by measuring [^{3}H]thymidine incorporation for 4 h. [^{3}H]thymidine (20 Ci/mmol) was added to the culture 20 h after the addition of IL-3 or SCF. In case of the measurement of the cell growth of co-cultured cells, the cells were starved for SCF for 6 h and then cultured in the presence of SCF and [^{3}H]thymidine for 4 h. The cellular radioactivity was measured by a liquid scintillation counter.

Counting of the Tissue Mast Cells

Thin ear sections (7 μm) were fixed with Carnoy's solution followed by staining with acidic toluidine blue in 0.6 N HCl for 5 s. The number of metachromatic cells was counted.

Mast Cell Reconstitution in the Tissues of the W/W^V Mice

BMMCs were injected into the peritoneal cavity (2×10^6 cells/mouse) or ear cutaneous tissues (1×10^6 cells/ear) of the *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice.

Statistical Analysis

Data are presented as the means ± s.e.m. Statistical significance for comparisons between groups was determined using Student's paired *t*-test or ANOVA. Additional comparisons were made with Dunnet's multiple comparison test for comparison with the control groups or the Tukey–Kramer multiple comparison test for all pairs of column comparison.

RESULTS

Induction of CD44 in Mast Cells Co-Cultured with Fibroblasts

Transcriptional upregulation of *CD44* was confirmed in BMMCs during the co-culture with Swiss 3T3 fibroblasts in the presence of SCF (Figure 1a). The CD44 induction was also confirmed at both the protein level and the cell surface expression level (Figure 1b and c). Although CD44 protein was also detected in fibroblasts, their expression levels were much lower than those in mast cells during the co-culture period (Figure 1b and data not shown).

HA-Dependent Cluster Formation of the Cultured Mast Cells

During the co-culture period, the majority of BMMCs were found to form clusters, which appeared around the third day after every subculture (Figure 2a). As Swiss 3T3 fibroblasts were reported to produce a set of extracellular matrix components including HA,[16] we speculated that HA is involved in the formation of these clusters. As expected, these clusters were rapidly and completely disrupted upon treatment with hyaluronidase (Figure 2b), indicating the essential role of HA in the cluster formation. Visualization of HA in the co-culture system using the biotinylated HA-binding protein (bHABP) revealed that HA formed fibrous networks, which developed in close proximity to the mast cells, which were immunoreactive to an anti-CD44 antibody (Figure 2c and d). Confocal microscopy showed that the HA network was bound to mast cells (Figure 2f–h). Three-dimensional images

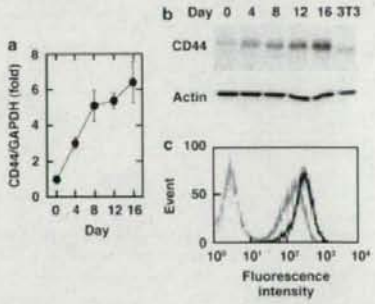
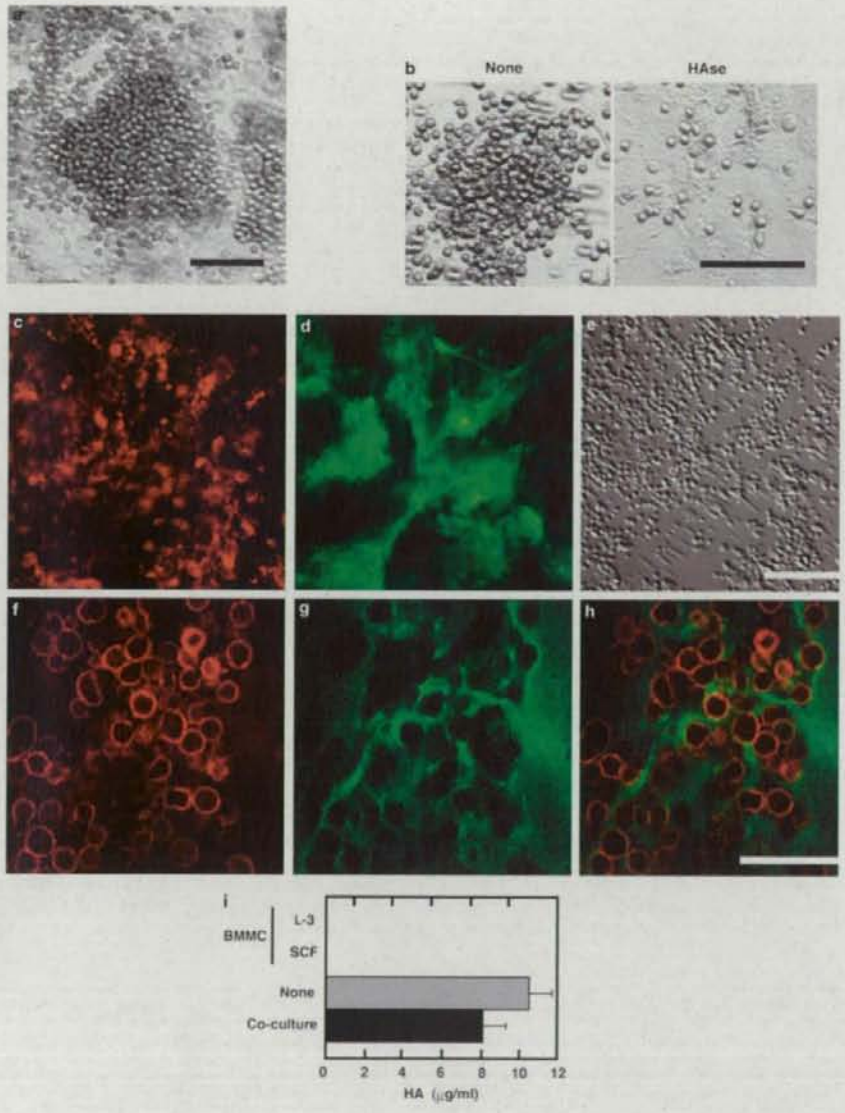


Figure 1 Induction of CD44 during co-culture with Swiss 3T3 fibroblasts. (**a**) Expression of CD44 mRNA in the co-cultured mast cells was evaluated by quantitative RT-PCR. The expression levels were normalized using those of *GAPDH*. The relative expression levels were presented as the fold expression. Values are presented as the means ± s.e.m. of three independent experiments. (**b**) Expression of CD44 at the protein levels in the co-cultured mast cells and Swiss 3T3 fibroblasts was determined by immunoblot analysis. (**c**) Surface expression of CD44 at day 0 (gray) and day 16 (black) was measured by flow cytometry with an anti-CD44 antibody (KM201, solid lines) and its isotype control antibody (dotted lines).


a
b
None
HAse
c
d
e
f
g
h
i
BMMC
L-3
SCF
None
Co-culture
0
2
4
6
8
10
12
HA (μg/ml)

revealed that the cluster was formed by the connection of individual mast cells by the extracellular matrix containing HA, and that the majority of mast cells were not homotypically aggregated (Supplementary Material 1). Although fluorescent dye-conjugated avidin was found to bind to mast cell granules,[17] no detectable signals were found in the experiments performed without bHABP (data not shown). We then identified the cells that produce HA in the co-culture system. A significant level of HA was detected in the day 4 co-culture medium and comparable to that in the medium of the single culture of Swiss 3T3 fibroblasts (Figure 2i). As HA was not detectable in the culture medium of BMMCs, we concluded that HA in the medium of the co-culture was produced exclusively by fibroblasts.

Impaired Proliferation of CD44 $^{-/-}$ BMMCs in the Co-Culture System

To determine the role of CD44 expressed in mast cells, we developed BMMCs from bone marrow cells of the *CD44*$^{-/-}$ mice and performed the co-culture experiments. There are no differences between *CD44*$^{+/+}$ and *CD44*$^{-/-}$ BMMCs in size, histamine content, or metachromasy of the acidic Toluidine blue staining (Figure 3a and data not shown). Furthermore, no significant difference was observed between *CD44*$^{+/+}$ and *CD44*$^{-/-}$ BMMCs in the surface expression of FcεRI or c-kit, both of which are typical surface markers of mast cells (Figure 3b). The CD44 deficiency did not change the [^{3}H]thymidine uptake and proliferation of BMMCs in response to IL-3 or SCF (Figure 3c and data not shown). These results indicate that commitment to mast cell lineage and cytokine-induced proliferative response are independent of CD44 in BMMCs, even although they express a low but detectable level of CD44.

In the co-culture system, the growth of *CD44*$^{-/-}$ mast cells was considerably impaired, in particular at the late phase (Figure 3d). In contrast to the continuous growth of control mast cells, the number of *CD44*$^{-/-}$ mast cells leveled off at day 8. In accord with this impaired growth, [^{3}H]thymidine uptake during the co-culture period was also decreased in *CD44*$^{-/-}$ mast cells (Figure 3e). On the contrary, granule maturation (Figure 3a, lower panels), compound 48/80-induced degranulation, or chymotryptic activity of mast cells (Supplementary Figure 1) was not affected by the CD44 deficiency, although the histamine content, tryptic activity, and carboxypeptidase A activity had the tendency to be augmented in *CD44*$^{-/-}$ mast cells (Supplementary Figure 1). These results suggested that CD44 supports the continuous growth of co-cultured mast cells, but has no or little influence on the phenotypic changes during the maturation period.

Effects of an Anti-CD44 Antibody on the Cluster Size and the Proliferation of Cultured Mast Cells

A series of studies have been performed using specific antibodies to determine the role of CD44. We investigated the levels of maturation and proliferation of the cultured mast cells in the presence of an anti-CD44 antibody, KM81, which was reported to block HA binding to CD44.[18] Proliferation of the co-cultured mast cells was significantly suppressed in the presence of KM81 (Figure 3f). On the other hand, KM81 did not affect the maturation indexes of mast cells, such as the Safranin-O staining, compound 48/80-induced degranulation, histamine content, or granule protease activities (Supplementary Figure 2). These results are consistent with the results obtained using *CD44*$^{-/-}$-cultured mast cells.

Treatment with KM81 did not affect proliferation of *CD44*$^{-/-}$ mast cells (Figure 3g), which were found to form only small clusters, the size or number of which was not changed in the presence of KM81 (Figure 3h). By contrast, the cluster size of *CD44*$^{+/+}$ cultured mast cells was reduced in the presence of KM81. Although co-cultured fibroblasts also express a low level of CD44, these results indicate that the primary target of KM81 is the CD44 expressed in mast cells.

Decrease in the Mast Cell Number in the CD44 $^{-/-}$ Mice

We then compared the two different CTMC populations, peritoneal and cutaneous mast cells, in the *CD44*$^{+/+}$ and *CD44*$^{-/-}$ mice. Flow cytometric analyses confirmed that the c-kit-positive cells in the peritoneal cavities and ear tissues of *CD44*$^{+/+}$ mice expressed not only FcεRI but also CD44 (Figure 4a), indicating that the mast cell population from these tissues express CD44. The expression levels of FcεRI and c-kit were comparable between the *CD44*$^{+/+}$ and *CD44*$^{-/-}$ mast cells (Figure 4a). The results of acidic toluidine blue staining indicated that the size and morphology of tissue mast cells were indistinguishable between *CD44*$^{+/+}$ and *CD44*$^{-/-}$ mice (Figure 4b–e). In contrast, both the number and the histamine content of mast cells were significantly lower in the *CD44*$^{-/-}$ mice than those in the wild-type mice (Figure 4f–i). The decrease in the number of tissue

Figure 2 HA-dependent cluster formation of the co-cultured mast cells. (**a**) A differential interference contrast microscopy (DIC) image of the co-cultured BMMCs at day 4 is presented. Bar = 100 μm. (**b**) Co-cultured cells at day 4 was treated with (HAse) or without (None) hyaluronidase. DIC images were obtained 5 min after the treatment. Bar = 100 μm. (**c**–**h**) The cells at day 4 in the co-culture system were fluorescently stained with an anti-CD44 antibody (**c** and **f**, red, KM81) and a biotinylated HA-binding protein (**d** and **g**, green). DIC images of the same fields were also obtained (**e**, the same eye field with **c** and **d**). Bar = 200 μm. The cells stained as described above were observed using the confocal laser-scanning microscopy (**f**–**h**). Bar = 50 μm. (**i**) The culture medium of Swiss 3T3 cells cultured alone (None) or together with BMMCs (co-culture) was recovered at day 4 and subjected to assay for HA concentration, which was also measured in the case that BMMCs were cultured in the presence of 10 ng/ml IL-3 (IL-3) or 100 ng/ml SCF (SCF). Values are presented as the means ± s.e.m. from at least three independent experiments.

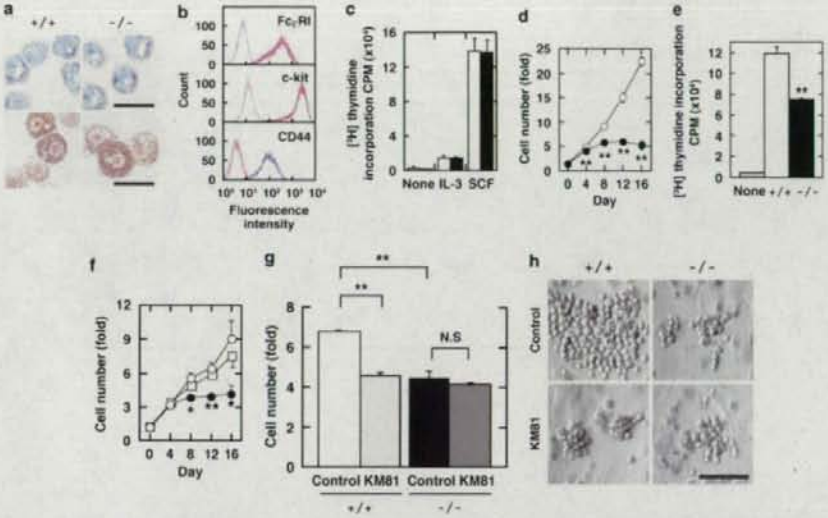


Figure 3 Involvement of CD44 in proliferation of the cultured mast cells. (**a**) Upper panels show the results of acidic toluidine blue staining of BMMCs derived from the $CD44^{+/+}$ and $CD44^{-/-}$ mice. Lower panels show the results of Alcian blue/Safranin-O staining of the cultured mast cells at day 16. Bar = 20 μm. (**b**) Surface expression levels of FcεRI, c-kit, and CD44 in $CD44^{+/+}$ (blue) or $CD44^{-/-}$ (red) BMMCs were measured by flow cytometry. Dotted lines show the profiles obtained using the isotype controls. (**c**) $CD44^{+/+}$ (open columns) or $CD44^{-/-}$ (closed columns) BMMCs were stimulated without (None) or with 10 ng/ml IL-3 (IL-3) or 100 ng/ml SCF (SCF). Proliferation of the cells were evaluated by the [^{3}H]thymidine uptake. Values were presented by the means ± s.e.m. of three independent experiments. (**d**) Profiles of the proliferation of $CD44^{+/+}$ (open circle) and $CD44^{-/-}$ (closed circle) mast cells during the co-culture period are presented. Values (the fold increase in the cell number) are presented as the means ± s.e.m. of three independent experiments. $^{**}P<0.01$ *vs* $CD44^{+/+}$ mast cells. (**e**) The [^{3}H]thymidine uptake was measured in mitomycin-treated 3T3 cells alone (None), co-culture with $CD44^{+/+}$ mast cells (+/+), and co-culture with $CD44^{-/-}$ mast cells (−/−). Values were presented by the means ± s.e.m. of three independent experiments. $^{**}P<0.01$ *vs* the co-culture with $CD44^{+/+}$ mast cells. (**f**) The $CD44^{+/+}$ BMMCs were co-cultured in the absence (open squares) or in the presence of an anti-CD44 antibody (5 μg/ml, closed circles, KM81) or its isotype control (open circles). The numbers of the co-cultured mast cells are presented. Values (the fold increase in the cell number) are presented as the means ± s.e.m. $^{**}P<0.01$ and $^{*}P<0.05$ *vs* the isotype control. (**g** and **h**) The number of the $CD44^{+/+}$ and $CD44^{-/-}$ co-cultured mast cells at day 8 were compared in the presence of the anti-CD44 antibody (5 μg/ml, KM81) or its isotype control (Control). Values are presented as the means ± s.e.m. of the three independent experiments. $^{**}P<0.01$ between two columns indicated. NS, not significant. DIC images were obtained at day 4 (**h**). Bar = 100 μm.

mast cells in the $CD44^{-/-}$ mice is consistent with the impaired growth of $CD44^{-/-}$ mast cells co-cultured with fibroblasts.

Requirement of CD44 for *In Vivo* Proliferation of the Mast Cells Reconstituted into Cutaneous Tissues of the $Kit^{W}/Kit^{W\text{-}v}$ Mice

As accumulating evidence has indicated that CD44 is involved in infiltration and chemotaxis of a variety of leukocytes, it remained ambiguous whether the decrease in the number of tissue mast cells in the $CD44^{-/-}$ mice resulted from the impaired cell growth or the defect in the migration of immature mast cells. We, therefore, investigated the proliferation of immature mast cells using an *in vivo* model system, in which BMMCs are reconstituted into the $Kit^{W}/Kit^{W\text{-}v}$ mice. BMMCs derived from the $CD44^{+/+}$ or $CD44^{-/-}$ littermates were injected into the peritoneal cavities or ear tissues of the $Kit^{W}/Kit^{W\text{-}v}$ mice, and the number and morphology of the transplanted BMMCs were examined 5 or 10 weeks after the initial injection. The transplanted BMMCs derived from $CD44^{+/+}$ and $CD44^{-/-}$ mice exhibited similar metachromasy in the peritoneal cavities and ear tissues (Figure 5a). In the peritoneal cavities, there was no difference in the number or histamine content of the transplanted mast cells derived from $CD44^{+/+}$ and $CD44^{-/-}$ mice (Figure 5b and c). On the other hand, the number of metachromatic cells upon toluidine blue staining in the tissues transplanted with $CD44^{+/+}$ BMMCs was significantly increased from 5 to 10 weeks after the initial injection, whereas that in the tissues transplanted with $CD44^{-/-}$ mast cells was unchanged

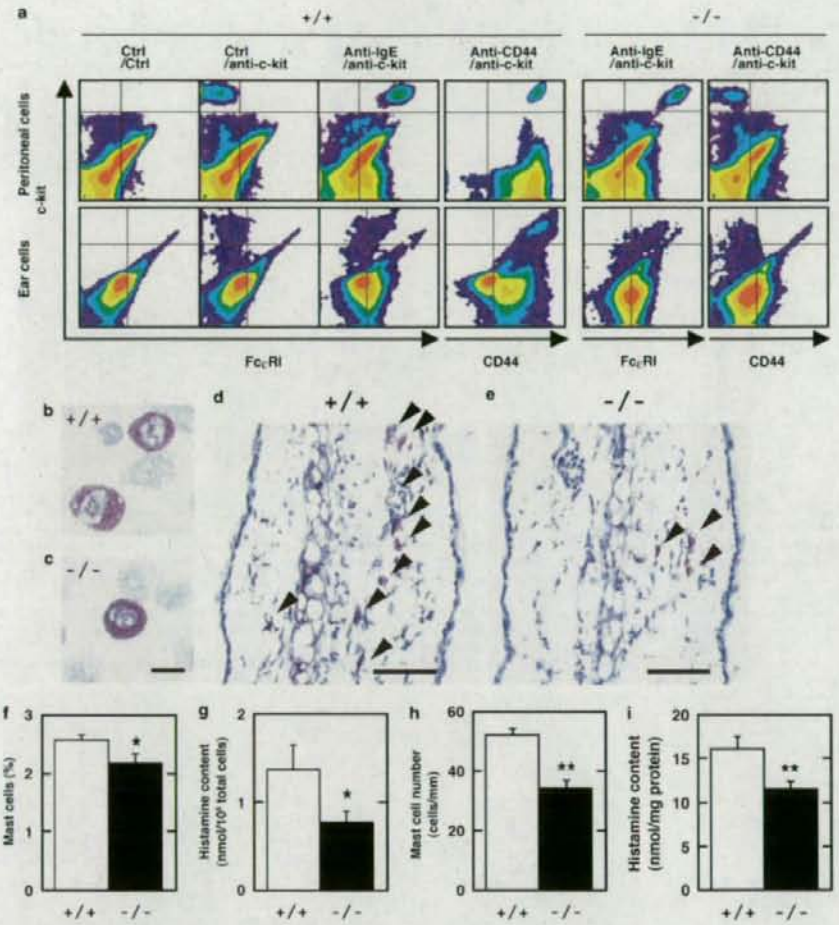


Figure 4 Decrease in the number of mast cells in the *CD44*$^{-/-}$ mice. (**a**) Surface expression levels of FcεRI, c-kit, and CD44 in the peritoneal cells, and the cells prepared from the ear tissues of the *CD44*$^{+/+}$ or *CD44*$^{-/-}$ mice were measured by flow cytometry. (**b**–**e**) The peritoneal cells (**b** and **c**) and ear tissues (**d** and **e**) of the *CD44*$^{+/+}$ (**b** and **d**) and *CD44*$^{-/-}$ (**c** and **e**) mice were stained with acidic toluidine blue. Bar = 10 μm (**b** and **c**) or 100 μm (**d** and **e**). (**d**) The ear mast cells are indicated by the arrowheads. (**f**) The percentages of mast cells in *CD44*$^{+/+}$ and *CD44*$^{-/-}$ peritoneal cells are presented ($n = 5$). (**g**) Histamine content in the peritoneal cells of the *CD44*$^{+/+}$ ($n = 11$) and *CD44*$^{-/-}$ ($n = 13$) mice was measured. (**h**) The number of mast cells in the ear tissues of *CD44*$^{+/+}$ and *CD44*$^{-/-}$ mice was measured ($n = 5$). (**i**) Histamine content in the ear tissues of *CD44*$^{+/+}$ ($n = 11$) and *CD44*$^{-/-}$ ($n = 13$) mice was measured. Values are presented as the means ± s.e.m. $^{*}P < 0.05$ and $^{**}P < 0.01$ (vs CD44$^{+/+}$) are regarded as significant.

(Figure 5d); the number of mast cells in the ear tissues transplanted with *CD44*$^{+/+}$ BMMCs was approximately twice as large as that in the tissues transplanted with *CD44*$^{-/-}$ BMMCs at 10 weeks after the injection. The histamine content in ear tissues at 10 weeks after the initial injection reflected the difference in the mast cell numbers (Figure 5e).

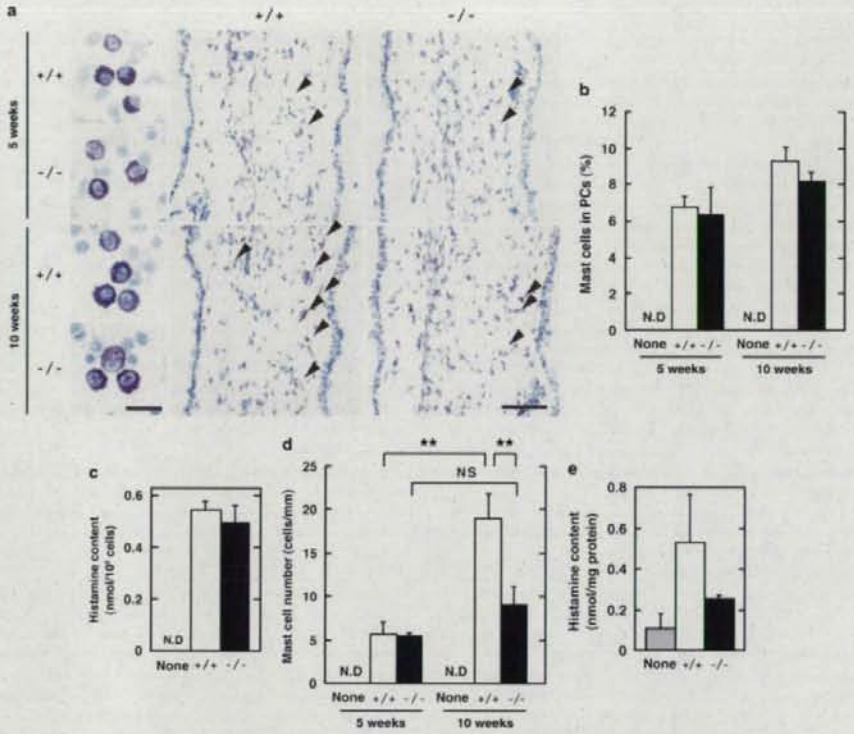


Figure 5 Impaired growth of transplanted *CD44*$^{-/-}$ mast cells in cutaneous tissues of the mast cell-deficient mice. BMMCs derived from the *CD44*$^{+/+}$ or *CD44*$^{-/-}$ littermate mice were injected in the peritoneal cavity or ear tissues of the *Kit*^{W}/*Kit*$^{W-v}$ mice. The number of the transplanted mast cells and histamine content were measured 5 or 10 weeks after the initial transplantation. (**a**) The peritoneal cells and ear tissues of the *Kit*^{W}/*Kit*$^{W-v}$ mice transplanted with *CD44*$^{+/+}$ (+/+) and *CD44*$^{-/-}$ (−/−) BMMCs were stained with acidic Toluidine blue. Bar = 20 μm (peritoneal cells) or 100 μm (ear tissues). The mast cells in the ear tissues are indicated by the arrowheads. (**b**) The percentages of mast cells in the peritoneal cells recovered from the *Kit*^{W}/*Kit*$^{W-v}$ mice transplanted without (None, $n = 3$) or with the *CD44*$^{+/+}$ (+/+, 5 weeks; $n = 7$, 10 weeks; $n = 8$) or *CD44*$^{-/-}$ (−/−, $n = 5$) BMMCs were presented. (**c**) Histamine contents in the peritoneal cells recovered from the *Kit*^{W}/*Kit*$^{W-v}$ mice transplanted without (None, $n = 3$) or with *CD44*$^{+/+}$ (+/+, $n = 8$) or *CD44*$^{-/-}$ (−/−, $n = 5$) BMMCs 10 weeks after the initial injection are presented. ND, not detectable. (**d**) The numbers of mast cells in the ear tissues from the *Kit*^{W}/*Kit*$^{W-v}$ mice transplanted without (None, 5 weeks; $n = 3$, 10 weeks; $n = 4$) or with *CD44*$^{+/+}$ (+/+, 5 weeks; $n = 6$, 10 weeks; $n = 5$) or *CD44*$^{-/-}$ (−/−, 5 weeks; $n = 7$, 10 weeks; $n = 3$) BMMCs are presented. Values of $^{**}P < 0.01$ are regarded as significant. NS, not significant. (**e**) Histamine contents in the ear tissues recovered from the *Kit*^{W}/*Kit*$^{W-v}$ mice transplanted without (None, $n = 4$) or with *CD44*$^{+/+}$ (+/+, $n = 5$) or *CD44*$^{-/-}$ (−/−, $n = 3$) BMMCs 10 weeks after the initial injection are presented. All values are presented as means ± s.e.m.

As the number of the transplanted mast cells in the spleen was found to be very small and similar between the mice injected with *CD44*$^{+/+}$ BMMCs and those with *CD44*$^{-/-}$ BMMCs (data not shown), it is less likely that the absence of CD44 affect the process of mast cell evasion from the injection sites. These results suggest that CD44 is involved in the regulation of mast cell number in cutaneous tissues.

DISCUSSION

Regulation of Tissue Mast Cell Number by CD44 Expressed in the Mast Cells

Recent studies identified the candidates for the committed mast cell progenitor, although the nature of them remains controversial.[19,20] As the mast cell progenitor is a rare population as compared with a large number of tissue

resident mast cells, proliferation after tissue infiltration of immature mast cells should be essential for maintenance of local mast cell number. Although proliferation of mast cells under emergency conditions, such as acute parasite infection, has been investigated in detail, it remains largely unknown how mast cell proliferation is regulated under steady-state conditions.[21] Our results obtained in the culture system indicated that the upregulation of CD44 during mast cell maturation should promote the proliferation of mast cells. The positive role of CD44 in mast cell proliferation was also supported by two *in vivo* findings, decrease in the number of tissue mast cells in the $CD44^{-/-}$ mice and the unchanged number of metachromatic cells in cutaneous tissues reconstituted with the $CD44^{-/-}$ BMMCs. As these *in vivo* data were obtained by the end point measurement, we cannot exclude possibilities that CD44 is involved in the process of maturation and/or survival of cutaneous mast cells. However, involvement of CD44 in maturation and survival of mast cells was not supported by the results obtained in the culture system. Our results collectively suggest that CD44 plays a crucial role in the regulation of mast cell number in the cutaneous tissues.

As accumulating evidence has indicated that CTMCs are involved in a diverse array of immune responses, it is possible that inflammatory responses in cutaneous tissues are affected by the decrease in mast cell number in the $CD44^{-/-}$ mice. CD44 is a potential therapeutic target for prevention of mast cells hyperplasia, which is often observed in chronic inflammatory diseases, such as atopic dermatitis and rheumatoid arthritis (RA). However, we could not detect significant changes in IgE-mediated immediate allergic responses in the $CD44^{-/-}$ mice (data not shown), such as passive cutaneous anaphylaxis reaction, which is solely dependent on cutaneous mast cells, raising the possibility that the mast cell number in the $CD44^{-/-}$ mice is enough to evoke such responses. We have to focus on the roles of CD44 under chronic inflammatory conditions, although multiple roles of CD44, such as extravasation and adhesion of the other leukocytes, would make difficult to ascertain the impact of CD44 expressed in mast cells.

We noticed the inconsistency in the number of peritoneal $CD44^{-/-}$ mast cells; as compared with the $CD44^{+/+}$ mice, the $CD44^{-/-}$ mice had a slightly but significantly fewer mast cells in the peritoneal cavity, whereas no significant differences were found between the number of transplanted $CD44^{+/+}$ and $CD44^{-/-}$ mast cells. This inconsistency may arise from the moderate increase in the number of the transplanted BMMCs in the peritoneal cavity in comparison with that in the cutaneous tissues. It is also possible that the degree of contribution of CD44 is lower in the peritoneal cavity than in cutaneous tissues as the extracellular matrix containing HA is not so much developed in the peritoneal cavity as in cutaneous tissues.

Regulation of CD44 by Tissue Environmental Factors

Alteration and breakdown of the extracellular matrix is a general feature of chronic inflammatory diseases. Recent studies have indicated that CTMCs are involved in inflammatory arthritis; mast cell-deficient mouse strains were resistant to autoimmune inflammatory arthritis;[22] and an increase in the local mast cell number is observed in RA and is closely correlated with the clinical progress of this disease.[23] The increase of mast cells in RA is attributed to the altered connection between mast cells and the extracellular matrix, as the synovial matrix in RA was often disrupted through altered metabolism of HA.[24] Human $CD34^+$ cell-derived cultured mast cells were reported to bind to HA through CD44 and to shed CD44 molecules upon activation of FcεRI, although it remained unknown what is the physiological roles of CD44 in human mast cells.[25] Shedding of CD44 may affect the fate of tissue mast cells that are activated upon FcεRI cross-linking. It is interesting to investigate the roles of CD44 in mast cells under chronic inflammatory conditions, where tissue mast cells are activated.

Mechanism Underlying CD44-Mediated Proliferation

Accumulating evidence has suggested that CD44 is involved in the proliferation of many cell types, although the underlying molecular mechanism remains largely unknown. Earlier studies indicated that CD44 can function as a co-receptor to activate the signaling pathway through interacting with several growth factor receptors.[26–28] It is possible that CD44 functions as a co-receptor to potentiate the growth signaling in mast cells. As c-kit is the dominant receptor that regulates proliferation and differentiation of mast cells, c-kit is one of the possible partners of CD44 when it functions as a co-receptor. Although we could not detect any interaction between CD44 and c-kit by immunoprecipitation (data not shown), further analyses are required about potential cross-talk of the signaling pathway between CD44 and c-kit.

CD44 was reported to modulate cytokine synthesis by leukocytes, such as T cells and macrophages,[7] raising a possibility that cytokine synthesis in mast cells are also modulated by CD44. An array of studies showed that mast cells are the sources of cytokines, which can affect their own growth in an autocrine manner.[21] CD44 and the extracellular matrix containing HA can trap and concentrate the growth factors and enzymes involved in the cell growth.[6] In-depth analyses of the matrix components may shed light on the roles of growth factors in mast cell proliferation in the clusters.

Tolg *et al*[29] showed that the receptor for HA-mediated motility (RHAMM), which is known as another HA receptor, functions as an essential regulator of the CD44-ERK1/2 axis in motogenic signaling in fibroblasts. Furthermore, Nedvetzki *et al*[30] showed that collagen-induced arthritis was exacerbated in the $CD44^{-/-}$ mice, and suggested that the signaling through RHAMM augmented by accumulated HA is responsible for the aggravation of joint inflammation. These findings indicate the co-operative and compensatory

functions of RHAMM. However, our attempts to show a possible involvement of RHAMM in the regulation of mast cell proliferation have been currently unsuccessful; no significant changes of proliferation were found in the presence of an anti-RHAMM antibody, although the surface expression of RHAMM was detected by flow cytometry (data not shown).

In summary, our results suggest that the number of mature mast cells in cutaneous tissues is regulated at least, in part, by CD44 in mice; CD44 promotes proliferation during the terminal differentiation of tissue mast cells. CD44 might be one of the potential therapeutic targets for chronic cutaneous inflammatory diseases accompanied by mast cell hyperplasia.

Supplementary Information accompanies the paper on the Laboratory Investigation website (http://www.laboratoryinvestigation.org)

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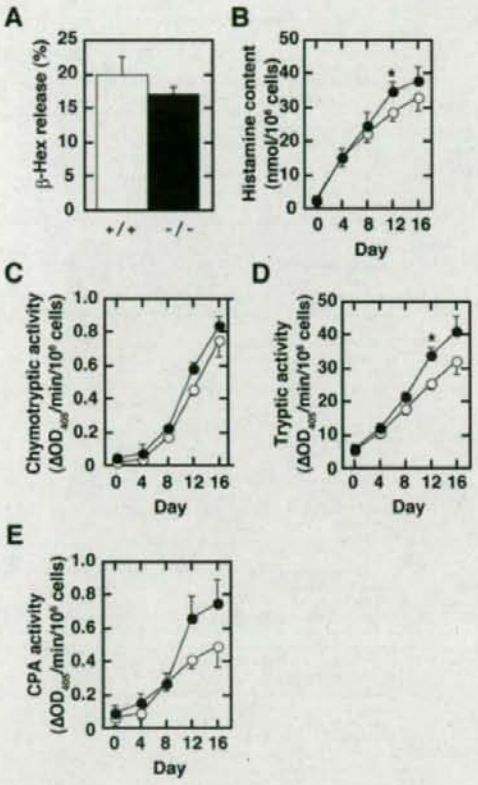
Supplemental figure 1

Normal maturation of the co-cultured *CD44*$^{-/-}$ mast cells.



Supplemental figure 2

Normal maturation of the cultured mast cells in the presence of an anti-CD44 antibody.