Mast cell deficiency in $Kit^{W\text{-}sh}$ mice does not impair antibody-mediated arthritis

Joseph S. Zhou, Wei Xing, Daniel S. Friend, K. Frank Austen, and Howard R. Katz

Department of Medicine, Harvard Medical School, and Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Boston, MA 02115

We previously reported that joint swelling, synovial thickening, and cartilage matrix depletion induced by the injection of anti-collagen monoclonal antibodies and lipopolysaccharide (LPS) in BALB/c mice are increased in the absence of inhibitory leukocyte immunoglobulin (Ig)-like receptor B4 (LILRB4; formerly gp49B1) in a neutrophil-dependent manner. Because both mast cells and neutrophils express LILRB4, we sought a mast cell requirement with mast cell–deficient mouse strains, but unexpectedly obtained full arthritis in $Kit^{W\text{-}sh}$ mice and full resistance in $Kit^{W/KitW\text{-}v}$ mice. $Kit^{W\text{-}sh}$ mice were indeed mast cell deficient as assessed by histology and the absence of IgE/mast cell–dependent passive cutaneous anaphylaxis in the ear and joint as well as passive systemic anaphylaxis. Deletion of LILRB4 in $Kit^{W\text{-}sh}$ mice exacerbated anti-collagen/LPS-induced joint swelling that was abolished by neutrophil depletion, establishing a counterregulatory role for LILRB4 in the absence of mast cells. Whereas blood neutrophil levels and LPS-elicited tissue neutrophilia were equal in $Kit^{W\text{-}sh}$ and Kit^{+} mice, both were impaired in $Kit^{W/KitW\text{-}v}$ mice. Although both strains are mast cell deficient and protected from IgE-mediated anaphylactic reactions, their dramatically different responses to autoantibody-mediated, neutrophil-dependent immune complex arthritis suggest that other host differences determine the extent of mast cell involvement. Thus, a conclusion for an absolute mast cell role in a pathobiologic process requires evidence from both strains.

CORRESPONDENCE
Howard R. Katz:
hkatz@rics.bwh.harvard.edu

In a neutrophil-dependent arthritis elicited by injections of a mixture of anti–type II collagen mAbs followed by LPS, mice lacking the tyrosine-based inhibitory receptor leukocyte Ig-like receptor B4 (LILRB4) have an exacerbated clinical response characterized morphologically by greater synovial thickening with neutrophil infiltration and depletion of articular cartilage matrix with erosions, compared with $Lilrb4^{+}$ mice (1). LILRB4 is expressed on and regulates pathobiologic functions of neutrophils in a vasculopathy model (2) and mast cells in anaphylaxis (3). Neutrophil infiltration in the arthritis model was greater in the affected joints of LILRB4 null ($Lilrb4^{-}$) mice compared with $Lilrb4^{+}$ mice, whereas the number and degranulation of synovial mast cells was not different in the two strains. However, the finding that $Lilrb4^{-}$ mice generate greater amounts of IL-1β, macrophage inflammatory protein 1α, and macrophage inflammatory protein 2 in the inflamed joints (1), each of which contributes to the tissue injury in this model, raises the possibility that mast cells might participate in a manner not revealed by numbers or degranulation, particularly because mast cells provide IL-1β during the initiating phase of inflammatory arthritis induced by injection of antibodies (Abs) to glucose 6-phosphate isomerase (GPI) (4). In the latter model, mast cell–deficient $Kit^{W}/Kit^{W\text{-}v}$ mice do not develop arthritis but are rendered susceptible by adoptive transfer of BM-derived mast cells from IL-$1\beta^{+}$ mice, but not from IL-$1\beta^{-}$ mice.

We report here the unexpected finding that $Kit^{W\text{-}sh}$ but not $Kit^{W}/Kit^{W\text{-}v}$ mice undergo full clinical and histologic arthritis induced by mAbs to type II collagen and LPS as compared with their respective Kit^{+} strains. Both strains are profoundly mast cell deficient and fail to exhibit mast cell–dependent hypersensitivity reactions. $Kit^{W}/Kit^{W\text{-}v}$ but not $Kit^{W\text{-}sh}$ mice had a basal neutropenia and deficient LPS-elicited neutrophilia, suggesting that the relative neutrophil deficiency in the $Kit^{W}/Kit^{W\text{-}v}$ strain may permit phenotypic complementation by mast cells. Anti-collagen/LPS-induced joint swelling was exacerbated in the absence of LILRB4 in the $Kit^{W\text{-}sh}$ strain and was neutrophil dependent in

The Journal of Experimental Medicine

both *Lilrb4*$^+$ and *Lilrb4*$^-$ mice in the *Kit*$^{W\text{-}sh}$ background. The ability to detect an effect of mast cell deficiency in *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice but not *Kit*$^{W\text{-}sh}$ mice suggests that conclusions about absolute mast cell dependence in multicomponent disease models such as mAb-mediated arthritis require confirmation in a mouse strain that is sufficient for the other key cellular elements.

RESULTS AND DISCUSSION

Mast cell deficiency in *Kit*$^{W\text{-}sh}$ mice does not prevent anti-collagen/LPS-induced arthritis

When *Kit*$^{W\text{-}sh}$ mice and mast cell–sufficient *Kit*$^+$ mice were injected with 2 mg of anti-collagen and 25 μg LPS 3 d later, joint swelling was detected in both strains on day 5, was maximal by day 6 with clinical scores of 9, and diminished to the baseline level by day 14 (Fig. 1 A). Furthermore, there were no significant differences in *Kit*$^+$ and *Kit*$^{W\text{-}sh}$ mice at day 7 in synovial thickness, cartilage matrix depletion, and synovial neutrophilia in ankle joints as assessed histologically (55 ± 5.4 vs. 52.7 ± 6.1 μm, 22.6 ± 2.2 vs. 17.0 ± 2.8% depletion, and 19.0 ± 6.1 vs. 21.2 ± 7.4 neutrophils/unit area; P = 0.8, 0.1, and 0.8, respectively; n = 9). Induction of less joint swelling by reducing the anti-collagen dose to 0.5 mg resulted in peak clinical scores on day 7 in *Kit*$^+$ and *Kit*$^{W\text{-}sh}$ mice of 2.3 ± 0.9 and 2.7 ± 0.9 (n = 3; P = 0.8), respectively, indicating that no effect of mast cell deficiency was uncovered even at the lower limit of clinical detection. Because we had expected a mast cell contribution based on studies reported in mast cell–deficient *Kit*^{W}/*Kit*$^{W\text{-}V}$ mice in the arthritis model induced with anti-GPI Abs (5), we evaluated our protocol in that strain and its *Kit*$^+$ control. When WBB6F1-*Kit*$^+$ mice were injected with 4 mg of anti-collagen and either 25 or 50 μg LPS, they developed joint swelling on day 5 and attained maximal clinical scores of 5 and 6.8, respectively, by day 7, whereas mast cell–deficient *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice did not exhibit joint swelling (Fig. 1 B).

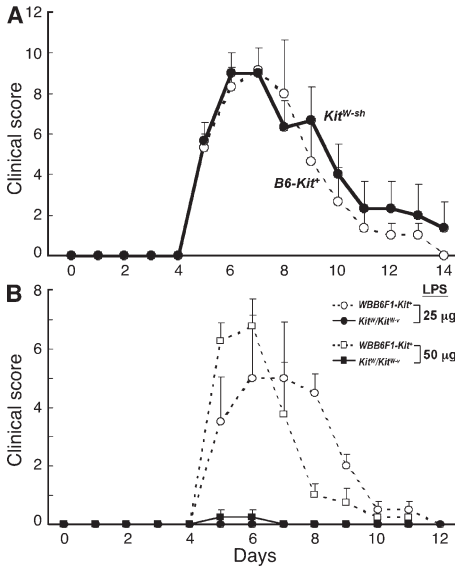

Figure 1. Anti-collagen/LPS-induced joint swelling in *Kit*$^{W\text{-}sh}$ and *Kit*^{W}/*Kit*$^{W\text{-}v}$ mice and their respective WT strains. Mice were injected i.v. with 2 mg of a mixture of four anti-collagen mAbs on day 0 and i.p. with 25 (A and B) or 50 (B) μg LPS on day 3. A score reflecting visual assessment of joint swelling in the paw, wrist, and ankle (described in Materials and methods) of each leg was assigned, and the four scores for each mouse were summed to yield the clinical score. Data are expressed as mean ± SEM; n = 6 from two experiments (days 0–7), n = 3 from one experiment (days 8–14) (A), n = 4 from one experiment with 25 μg LPS, and n = 4 from one experiment with 50 μg LPS (B).

Histologic and functional evidence of mast cell deficiency in *Kit*$^{W\text{-}sh}$ mice

We confirmed that our *Kit*$^{W\text{-}sh}$ mice were mast cell deficient by histologic and functional criteria at the age range at which arthritis was induced. As determined in tissue sections stained for chloroacetate esterase (CAE) activity, there were essentially no mast cells in the synovium and subsynovium of the ankle joint, tongue, spleen, liver, kidney, heart, or intestine of 5–9-wk-old naive *Kit*$^{W\text{-}sh}$ mice, and the number of mast cells in the ear skin and the dermis adjacent to the subsynovium of the ankle joint was 1.7 and 3.4%, respectively, of the number in *Kit*$^+$ mice (n = 4–7). Estimates of the number of mast cells in the ear and back skin of 10-wk-old *Kit*$^{W\text{-}sh}$ mice have ranged from 6–10% of that of *Kit*$^+$ mice, as assessed by alcian blue (6) or toluidine blue staining (7) of tissue sections, to not detectable in the ear skin by methylene blue staining or CAE activity (8). *Kit*$^+$ but not *Kit*$^{W\text{-}sh}$ mice exhibited passive cutaneous anaphylaxis (PCA) reactions in the ear and paw as measured by extravasation of Evans Blue dye-bearing protein (Fig. 2), indicating that the *Kit*$^{W\text{-}sh}$ mice are functionally deficient in dermal mast cells. Finally, to exclude a pool of functional mast cells at any site, we subjected *Kit*$^{W\text{-}sh}$ and *Kit*$^+$ mice to passive systemic anaphylaxis induced by i.v. injection of anti–mouse IgE. Four out of four *Kit*$^+$ mice became moribund, whereas there was no clinical response in four out of four *Kit*$^{W\text{-}sh}$ mice. Thus, the *Kit*$^{W\text{-}sh}$ strain is indistinguishable from the *Kit*^{W}/*Kit*$^{W\text{-}v}$ strain with regard to mast

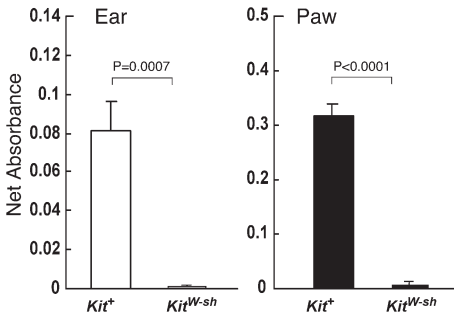

Figure 2. IgE-PCA in mast cell–deficient and –sufficient mice. Mice were injected i.d. in one ear or s.c. in one paw with 25 and 75 ng of mouse monoclonal IgE anti-DNP, respectively, and were injected in the other ear or paw with saline. After 20 h, the mice were injected i.v. with 100 μg DNP-HSA/1% Evans blue. After 0.5 h, mice were killed, their ears and paws were excised, and the Evans blue was extracted with formamide and quantified by spectrophotometry. Data are expressed as mean ± SEM; n = 7–9 from two experiments (ear) and n = 5 from three experiments (paw).

cell deficiency by morphological and IgE-dependent functional criteria. The functionally equivalent mast cell deficiency of $Kit^{W\text{-}sh}$ and $Kit^{W}/Kit^{W\text{-}v}$ mice had already been observed in models of antigen-induced pulmonary inflammation and venom detoxification, with the latter reflecting the ability of mast cell carboxypeptidase A to degrade sarafotoxins in venom (9–11).

Basal and LPS-induced elevation of circulating and tissue neutrophils differs in mast cell–deficient strains

Because neutrophils are essential for the generation of joint swelling and tissue pathology in the anti-collagen/LPS and anti-GPI models (1, 12), we quantified the number of peripheral blood neutrophils in $Kit^{W}/Kit^{W\text{-}v}$ and $Kit^{W\text{-}sh}$ mice and their respective Kit^{+} control strains before and 24 h after i.p. injection of 25 μg LPS, the dose used in the anti-collagen/LPS model. The concentration of peripheral blood neutrophils in 7–9-wk-old naive $Kit^{W}/Kit^{W\text{-}v}$ mice was only one third of that in WBB6F1-Kit^{+} mice (Fig. 3). LPS induced a significant threefold increase in the concentration of peripheral blood neutrophils in both of these strains, and the level reached in $Kit^{W}/Kit^{W\text{-}v}$ mice was again only one third of that in WBB6F1-Kit^{+} mice. Because there was no arthritis in the $Kit^{W}/Kit^{W\text{-}v}$ strain, we compared the LPS-elicited efflux of peripheral blood neutrophils into the ear skin of $Kit^{W}/Kit^{W\text{-}v}$ and WBB6F1-Kit^{+} mice. 24 h after intradermal (i.d.) injection of 50 μg LPS, there were significantly fewer neutrophils in the peripheral blood of $Kit^{W}/Kit^{W\text{-}v}$ mice compared with WBB6F1-Kit^{+} mice (0.68 ± 0.074 vs. $1.5 \pm 0.15 \times 10^3$ cells per μl, respectively; $n = 9$–11; $P = 0.0002$) and recruited into the ears (5.8 ± 0.8 vs. 13.3 ± 1.7 neutrophils per unit length, respectively; $n = 11$–12; $P = 0.008$). Our data confirm and extend the findings of Chervenick and Boggs reported in 1969 (13), which indicated that the concentration of peripheral blood neutrophils in naive 3-mo-old $Kit^{W}/Kit^{W\text{-}v}$ mice (the youngest mice examined) was a significantly lower 31% of that of WBB6F1-Kit^{+} mice, and reached ∼80% of normal at 6 mo. The number of BM neutrophils in $Kit^{W}/Kit^{W\text{-}v}$ mice was a significantly lower 52–64% of that of WBB6F1-Kit^{+} mice from 3 to 6 mo of age. Even in older $Kit^{W}/Kit^{W\text{-}v}$ mice with near normal peripheral blood neutrophil levels, the increase in neutrophils 6 h after i.p. injection of 5 μg LPS was only ∼60% of that of WBB6F1-Kit^{+} mice (13). Naive $Kitl^{Sl}/Kitl^{Sl\text{-}d}$ mice that are on the WCB6F1 background and are stem cell factor deficient (14) also have a BM and peripheral blood neutropenia (15).

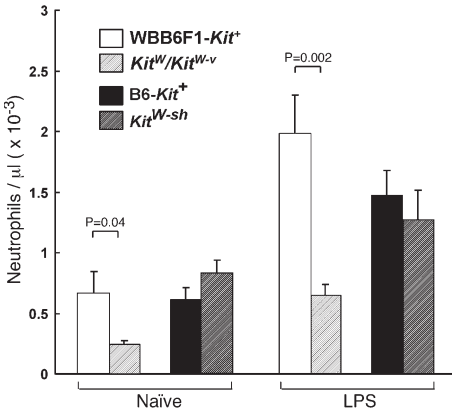

Figure 3. Peripheral blood neutrophil concentrations in naive and LPS-treated mast cell–deficient and –sufficient mice. Blood samples were obtained from $Kit^{W}/Kit^{W\text{-}v}$ and $Kit^{W\text{-}sh}$ mice and their respective control strains before and 24 h after i.p. injection of saline or 25 μg LPS. The concentration of neutrophils in the samples was quantified with an automated cell counter. Data are expressed as mean ± SEM; $n = 7$–15 per genotype.

In contrast with $Kit^{W}/Kit^{W\text{-}v}$ mice, the concentration of neutrophils in the blood of naive 7–9-wk-old $Kit^{W\text{-}sh}$ mice was not different than that of Kit^{+} controls (Fig. 3). Injection of 25 μg LPS i.p. induced comparable increases in the concentration of peripheral blood neutrophils after 24 h in Kit^{+} and $Kit^{W\text{-}sh}$ mice, respectively (Fig. 3). 24 h after i.d. injection of 50 μg LPS, there was no significant difference in the number of neutrophils in the blood of Kit^{+} and $Kit^{W\text{-}sh}$ mice (2.0 ± 0.21 vs. $1.9 \pm 0.21 \times 10^3$ cells per μl, respectively; $n = 3$–4) or migration of neutrophils into the ears (13.9 ± 1.2 vs. 12.2 ± 3.5 neutrophils per unit length, respectively; $n = 6$–8; $P = 0.7$). Hence, unlike $Kit^{W}/Kit^{W\text{-}v}$ mice, $Kit^{W\text{-}sh}$ mice exhibit the same constitutive blood level and LPS-induced tissue recruitment of neutrophils as their Kit^{+} controls. The cumulative findings suggest a role for the stem cell factor–Kit interaction in neutrophil development in certain genetic backgrounds and/or when mutations in that ligand–receptor pair are located within the molecules themselves (14, 16) and hence are global (i.e., $Kit^{W}/Kit^{W\text{-}v}$ and $Kit^{Sl}/Kit^{Sl\text{-}d}$ mice in the rather unusual WBB6F1 and WCBF1 backgrounds, respectively). In contrast, an alteration upstream of the *Kit* gene (17, 18) is associated with a selective deficiency of expression in mast cells and probably not in myeloid progenitors that populate the neutrophil lineage (i.e., $Kit^{W\text{-}sh}$ mice in the C57BL/6 background). The neutropenia is not the only difference in the two strains, as $Kit^{W}/Kit^{W\text{-}v}$ but not $Kit^{W\text{-}sh}$ mice have a macrocytic anemia and deficiency in γδ T cells (7, 19), whereas both strains have deficiencies in melanocytes (20) and interstitial cells of Cajal (7). Because the absence of mast cells in $Kit^{W\text{-}sh}$ mice resulted from a 2-cM inversion upstream of the *Kit* gene in C3H/HeJ × 101/H F1 mice (19, 21), it is possible that the retention of certain alleles from the F1 strain even after backcrossing into C57BL6 mice contributes to the phenotypic differences of $Kit^{W\text{-}sh}$ mice compared with $Kit^{W}/Kit^{W\text{-}v}$ mice.

Splenic, but not BM, myeloid hyperplasia in $Kit^{W\text{-}sh}$ mice

As part of a general histologic assessment for tissue mast cells, we noted that the spleens of $Kit^{W\text{-}sh}$ mice as compared with their Kit^{+} controls had a significant 2.4-fold greater number of CAE^{+} cells with myeloid morphology (Fig. 4 A) that had a primarily subcapsular localization and included a significantly greater percentage of immature forms ($68 \pm 7\%$ and $16 \pm 4\%$, respectively; $n = 3$; $P = 0.004$). Induction of synovitis with anti-collagen/LPS caused an increase in the splenic CAE^{+} cells in both strains that reached a significantly greater level in the $Kit^{W\text{-}sh}$ mice (Fig. 4 A). As measured by flow cytometry,

dispersed splenocytes from naive Kit^{+} and $Kit^{W\text{-}sh}$ mice were 7 ± 2 and 32 ± 7% Gr-1^{+}, respectively (n = 4; P = 0.01; representative histograms are presented in Fig. 4 B), consistent with the greater number of CAE^{+} myeloid cells in $Kit^{W\text{-}sh}$ mice. In contrast, there was no difference in the number of CAE^{+} cells in the BM of Kit^{+} and $Kit^{W\text{-}sh}$ mice (64 ±6.5 and 70 ± 1.2 cells per high power field, respectively; n = 4). There was also no difference in the number of BM cells recovered from the femurs and tibias of Kit^{+} and $Kit^{W\text{-}sh}$ mice (5.4 ± 1.5 and 5.8 ± 1.4 × 10^{7}, cells/mouse, respectively; n = 3) or in the percentage of Gr-1^{+} cells in the BM (72 ± 8 and 77 ± 5%, respectively; n = 3–4). Because the BM of $Kit^{W\text{-}sh}$ mice lacked these changes, and the blood level and recruitment of neutrophils in the joints and ears of $Kit^{W\text{-}sh}$ mice was the same as in Kit^{+} mice with no increase in morphologically immature myeloid cells, there was no evidence that the splenic dysmyelopoiesis was a factor in the normal response to anti-collagen/LPS-induced arthritis in mast cell–deficient $Kit^{W\text{-}sh}$ mice. Indeed, there was no difference in Kit^{+} and $Kit^{W\text{-}sh}$ mice at day 7 of the anti-collagen mAbs/LPS model in the number of peripheral blood neutrophils (1.9 ± 0.7 and 1.5 ± 0.5 × 10^{3}/μl, respectively; n = 4; P = 0.6) and neutrophils per unit area in the synovium (19.6 ± 6.8 and 21.2 ± 7.4 cells/unit area, respectively; n = 9; P = 0.8) and joint space (5.8 ± 1 and 8.4 ± 2.6 cells/unit area, respectively; n = 9; P = 0.3) at day 7.

Inflammatory arthritis is exacerbated in $Lilrb4^{-}/Kit^{W\text{-}sh}$ mice

The full inflammatory arthritis in $Kit^{W\text{-}sh}$ mice afforded the opportunity to assess the potential contribution of LILRB4 in the absence of mast cells. The clinical scores in the $Lilrb4^{+}/Kit^{W\text{-}sh}$ and $Lilrb4^{-}/Kit^{W\text{-}sh}$ on day 7 reached values of 6.1 ± 1.4 and 8.7 ± 1.6, respectively, and the entire time course of clinical scores in $Lilrb4^{-}/Kit^{W\text{-}sh}$ mice was significantly greater than in $Lilrb4^{+}/Kit^{W\text{-}sh}$ mice (P = 0.03; Fig. 5). Histologic analysis of the synovial thickness in the talo-tibial articulation of the ankle joint at day 7 revealed a trend toward greater thickness in $Lilrb4^{-}/Kit^{W\text{-}sh}$ mice compared with $Lilrb4^{+}/Kit^{W\text{-}sh}$ mice (71.0 ± 10 vs. 52.7 ± 6.1 μm, respectively; n = 9; P = 0.1; the measurements were made in the same experiments that provided the comparison of Kit^{+} and $Kit^{W\text{-}sh}$ mice noted above). Moreover, as assessed by digital image analysis of the loss of staining with safranin O, there was a significant approximately twofold greater depletion of cartilage matrix at day 7 in $Lilrb4^{-}/Kit^{W\text{-}sh}$ mice compared with $Lilrb4^{+}/Kit^{W\text{-}sh}$ mice (32.2 ± 6.3 vs. 17.0 ± 2.8%; n = 9; P = 0.04). Hence, the exacerbation of these characteristics in the absence of LILRB4 proceeds without a mast cell influence, compatible with a central role for neutrophils.

To establish an absolute neutrophil requirement in the LILRB4-sufficient and -deficient $Kit^{W\text{-}sh}$ strains, mice were injected i.p. with anti–Gr-1 on days 2 and 4 of the anti-collagen/LPS protocol, as done previously with $Lilrb4^{+}$ and $Lilrb4^{-}$ mast cell–sufficient mice on the BALB/c background (1). Treatment with anti–Gr-1 depleted peripheral blood neutrophils by >95% compared with untreated mice and suppressed clinical scores in both $Lilrb4^{+}/Kit^{W\text{-}sh}$ (0 ± 0 vs. 6.0 ± 3, respectively) and $Lilrb4^{-}/Kit^{W\text{-}sh}$ mice (0 ± 0 vs. 9.0 ± 0) at day 7 (n = 2–5 mice per genotype).

In addressing the discrepancy as to why the $Kit^{W\text{-}sh}$ but not $Kit^{W}/Kit^{W\text{-}v}$ mast cell–deficient strain is fully susceptible to anti-collagen/LPS-induced arthritis, we confirmed an old observation that $Kit^{W}/Kit^{W\text{-}v}$ mice are neutropenic and mobilize blood neutrophils poorly (13). Although there are also deficiencies in other lineages in $Kit^{W}/Kit^{W\text{-}v}$ mice that are not present in $Kit^{W\text{-}sh}$ mice, we have focused on the neutrophil because of its prominence in the model. This has recently been highlighted in studies of arthritis induced with Ab to GPI in which clinical and tissue pathology was interrupted when the number of neutrophils fell due to blockade of their continuous recruitment from blood by local generation or action of leukotriene B_4 with inhibitors of 5-lipoxygenase or

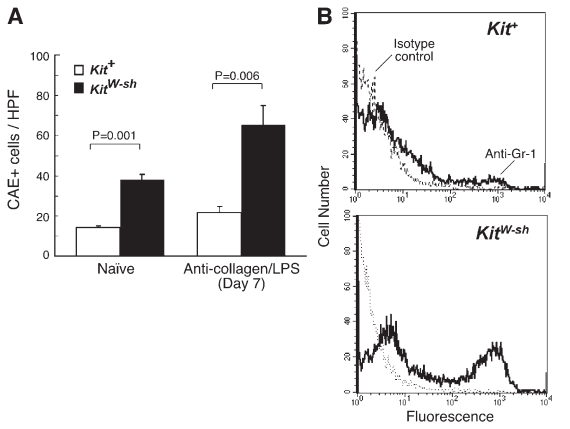

Figure 4. Histologic and flow cytometric assessments of myeloid hyperplasia in the spleen of $Kit^{W\text{-}sh}$ mice. (A) Tissue sections from naive mice and mice on day 7 after injection of anti-collagen/LPS were stained for CAE activity and counterstained with hematoxylin. The number of CAE^{+} cells in the subcapsular region was counted in five 40× fields per sample. Data are expressed as mean ± SEM; n = 5 (naive) and n = 6 (day 7). (B) Spleen cells were stained with PE-labeled anti–Gr-1 or an isotype-matched negative control mAb, and fluorescence was analyzed by flow cytometry.

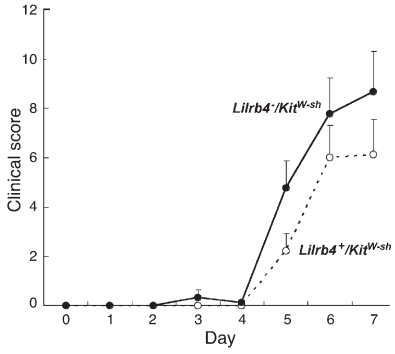

Figure 5. Anti-collagen/LPS-induced joint swelling in $Kit^{W\text{-}sh}$ mice sufficient and deficient in LILRB4. Mice were injected and their clinical scores were determined as described in the legend for Fig. 1, except that 1 mg of anti-collagen was used. Data are expressed as mean ± SEM; n = 9 from three experiments.

the BLT_1 receptor (22, 23). The finding that a critical concentration of neutrophils must be achieved to control the growth of bacteria in tissue (24) may be relevant to the requirement for presence of a sufficient concentration of neutrophils to induce inflammation and tissue pathology in the arthritis models. That the absence of the control receptor LILRB4 in mast cell–deficient $Kit^{W\text{-}sh}$ mice augments the clinical and tissue pathology in the anti-collagen/LPS model again supports the importance of neutrophils as opposed to mast cells, but does not exclude a contribution by other cell types lacking the receptor. Our findings do not question a role for adoptively transferred WT BM-derived mast cells in uncovering the arthritic potential of mAb in the $Kit^{W}/Kit^{W\text{-}v}$ strain as shown in the anti-GPI model (5), but rather suggest that the limitation of other cell types, such as neutrophils, allows recognition of a mast cell contribution. Indeed, engraftment of mast cells into $Kit^{W}/Kit^{W\text{-}v}$ mice corrects a deficiency in the influx of peritoneal neutrophils in response to i.p. injection of peptidoglycan (25). Naive WBB6F1-Kit^{+} mice have six times the number of peritoneal mast cells as C57BL/6-Kit^{+} mice, and 24 h after cecal ligation and puncture have 20 times the number of peritoneal neutrophils (26). The propensity for elevated numbers of mast cells and neutrophils in WBB6F1-Kit^{+} mice combined with the neutropenia in mast cell–deficient $Kit^{W}/Kit^{W\text{-}v}$ mice may magnify the contribution of mast cells to neutrophil-dependent models of inflammation in this strain compared with the C57BL/6 strain. Hence, our findings suggest that a potential role for mast cells is not always critical, and that the availability of a second mast cell–deficient strain allows separation of absolute and relative roles for mast cells in disease models.

MATERIALS AND METHODS

Mice. $Lilrb4^{+/-}$ mice on a mixed 129/BALB/c background (3) were backcrossed to B6 mice five times, and for the last four of those backcrosses, mice with the greatest number of B6 alleles were selected by microsatellite analyses (Charles River Laboratories). The resulting backcrossed $Lilrb4^{+/-}$ mice were intercrossed, and their homozygous progeny were bred in parallel to yield $Lilrb4^{+}$ and $Lilrb4^{-}$ mice. $Kit^{W\text{-}sh}$ mice, backcrossed at least 10 times to the B6 background, were obtained via D. Lee (Brigham and Women's Hospital, Boston, MA) from P. Wolters and G. Caughey (University of California, San Francisco, San Francisco, CA) (27), who obtained them from P. Besmer (Memorial Sloan-Kettering Cancer Center, New York, NY). The mice were bred with $Lilrb4^{-}$ mice to yield $Lilrb4^{+/-}/Kit^{+/W\text{-}sh}$ mice, which were intercrossed to produce $Lilrb4^{+}/Kit^{W\text{-}sh}$ and $Lilrb4^{-}/Kit^{W\text{-}sh}$ mice. 7–9-wk-old male mice were used for experiments. Mice were maintained in a specific pathogen-free barrier facility at the Dana-Farber Cancer Institute, and the studies were approved by the Animal Care and Use Committee.

Induction of proliferative synovitis. Mice were injected i.v. with a mixture of anti–type II collagen mAbs (Chemicon International Inc.), followed 3 d later by an i.p. injection of LPS (*Escherichia coli* O111:B4; Chemicon) (1, 28) at the doses indicated in the Results. Because the intensity of clinical pathology obtained with different batches of anti-collagen varied, doses were chosen that provided an average peak clinical score (described below) of at least 5.

Clinical and histologic analyses. A clinical score for joint swelling in each leg was assigned according to the following scale: 0, no detectable swelling; 1, swelling in metatarsal phalange joints, an individual phalanx, or local edema; 2, swelling localized to either the dorsal or ventral surface of a paw; 3, swelling on all aspects of a paw (1). The sum of the four scores was defined as the clinical score for each mouse. Synovial thickness was measured in histologic sections of the talo-tibial articulation of the ankle joint stained for CAE activity as described previously (1). Cartilage matrix depletion was measured in sections stained with Safranin O as described previously (1), except that the areas of cartilage matrix depletion within the cartilaginous regions were outlined in digital photomicrographs, converted to pixels with ImageJ software (Image Processing and Analysis in Java developed by the National Institutes of Health), and expressed as a percentage of the total cartilaginous region. Quantification of LPS-induced neutrophilia was performed in ear sections stained for CAE activity (2).

IgE-dependent anaphylaxis. For PCA in the ear, mice were injected i.d. in one ear with 25 ng IgE anti-DNP (SPE-7; Sigma-Aldrich) and with an equal volume of saline (20 μl) in the other ear. 20 h later, mice were injected i.v. with 100 μg DNP-HSA (30–40 moles DNP/HSA; Sigma-Aldrich) in 100 μl of 1% Evans Blue dye. After 0.5 h, mice were killed, ear tissue was obtained with a 6-mm diameter punch, dye was extracted from the tissue by incubation in 200 μl of formamide at 58°C for 48 h, the tissue was pelleted by centrifugation, and the dye in the supernatant was quantified spectrophotometrically (3, 29). For PCA in the paw, mice were injected s.c. in one hind paw with 75 ng IgE anti-DNP and in the other hind paw with an equal volume (10 μl) of saline. Mice were challenged with antigen and Evans blue, killed, and the paws were collected from the fur line and cut into small pieces. The dye was extracted as for PCA in the ear except that an extraction volume of 1 ml was used. For IgE-dependent systemic anaphylaxis, mice were injected i.v. with 40 μg of rat anti–mouse IgE (R35-72; BD Biosciences).

Peripheral blood neutrophil counts and Ab-mediated depletion. Blood was collected, and leukocyte counts and differentials were determined with an automated cell counter as described previously (1). For neutrophil depletion, mice were injected i.p. with rat anti–Gr-1 mAb or rat IgG2b isotype control mAb (1, 12).

Flow cytometry. Spleens were disaggregated mechanically to liberate splenocytes, and the cells were incubated for 10 min at room temperature with ACK buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 111 μM disodium EDTA in Milli-Q water) to lyse erythrocytes, centrifuged, and resuspended at 10^6 cells/ml in HBA buffer (calcium- and magnesium-free HBSS containing 0.1% [wt/vol] BSA and 0.02% [wt/vol] sodium azide). The spleen cells were incubated for 30 min at 4°C with a saturating concentration of PE-labeled anti–Gr-1 or an equal concentration of isotype-matched negative control rat IgG2b mAb, washed in HBA buffer twice, and analyzed for fluorescence intensity on a FACSort (Becton Dickinson Immunocytometry Systems).

Statistical analyses. Data are expressed as mean ± SEM unless otherwise noted. Differences in single measurements between groups of mice were assessed with Student's unpaired, two-tailed *t* test. Differences in the time courses of clinical scores were assessed with ANOVA. p-values of <0.05 were defined as statistically significant.

The authors thank Juying Lai for technical assistance.

This work was supported by grants AI-31599, AI-41144, and HL-36110 from the National Institutes of Health and a grant from the Arthritis Foundation.

The authors have no conflicting financial interests.

Submitted: 9 July 2007
Accepted: 18 October 2007

REFERENCES

1. Zhou, J.S., D.S. Friend, D.M. Lee, L. Li, K.F. Austen, and H.R. Katz. 2005. gp49B1 deficiency is associated with increases in cytokine and chemokine production and severity of proliferative synovitis induced by anti-type II collagen mAbs. *Eur. J. Immunol.* 35:1530–1538.

2. Zhou, J.S., D.S. Friend, A.F. Feldweg, M. Daheshia, L. Li, K.F. Austen, and H.R. Katz. 2003. Prevention of lipopolysaccharide-induced microangiopathy by gp49B1: evidence for an important role for gp49B1 expression on neutrophils. *J. Exp. Med.* 198:1243–1251.
3. Daheshia, M., D.S. Friend, M.J. Grusby, K.F. Austen, and H.R. Katz. 2001. Increased severity of local and systemic anaphylactic reactions in gp49B1-deficient mice. *J. Exp. Med.* 194:227–233.
4. Nigrovic, P.A., B.A. Binstadt, P.A. Monach, A. Johnsen, M. Gurish, Y. Iwakura, C. Benoist, D. Mathis, and D.M. Lee. 2007. Mast cells contribute to initiation of autoantibody-mediated arthritis via IL-1. *Proc. Natl. Acad. Sci. USA.* 104:2325–2330.
5. Lee, D.M., D.S. Friend, M.F. Gurish, C. Benoist, D. Mathis, and M.B. Brenner. 2002. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science.* 297:1689–1692.
6. Yamazaki, M., T. Tsujimura, E. Morii, K. Isozaki, H. Onoue, S. Nomura, and Y. Kitamura. 1994. C-*kit* gene is expressed by skin mast cells in embryos but not in puppies of Wsh/Wsh mice: age-dependent abolishment of c-kit gene expression. *Blood.* 83:3509–3516.
7. Grimbaldeston, M.A., C.C. Chen, A.M. Piliponsky, M. Tsai, S.Y. Tam, and S.J. Galli. 2005. Mast cell-deficient *W-sash* c-*kit* mutant $Kit^{W\text{-}sh}/^{W\text{-}sh}$ mice as a model for investigating mast cell biology *in vivo*. *Am. J. Pathol.* 167:835–848.
8. Wolters, P.J., J. Mallen-St Clair, C.C. Lewis, S.A. Villalta, P. Baluk, D.J. Erle, and G.H. Caughey. 2005. Tissue-selective mast cell reconstitution and differential lung gene expression in mast cell-deficient $Kit^{W\text{-}sh}/Kit^{W\text{-}sh}$ sash mice. *Clin. Exp. Allergy.* 35:82–88.
9. Yu, M., M. Tsai, S.Y. Tam, C. Jones, J. Zehnder, and S.J. Galli. 2006. Mast cells can promote the development of multiple features of chronic asthma in mice. *J. Clin. Invest.* 116:1633–1641.
10. Metz, M., A.M. Piliponsky, C.C. Chen, V. Lammel, M. Abrink, G. Pejler, M. Tsai, and S.J. Galli. 2006. Mast cells can enhance resistance to snake and honeybee venoms. *Science.* 313:526–530.
11. Schneider, L.A., S.M. Schlenner, T.B. Feyerabend, M. Wunderlin, and H.-R. Rodewald. 2007. Molecular mechanism of mast cell–mediated innate defense against endothelin and snake venom sarafotoxin. *J. Exp. Med.* 204:2629–2639.
12. Wipke, B.T., and P.M. Allen. 2001. Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. *J. Immunol.* 167:1601–1608.
13. Chervenick, P.A., and D.R. Boggs. 1969. Decreased neutrophils and megakaryocytes in anemic mice of genotype W/W^{v}. *J. Cell. Physiol.* 73:25–30.
14. Flanagan, J.G., D.C. Chan, and P. Leder. 1991. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the Sld mutant. *Cell.* 64:1025–1035.
15. Ruscetti, F.W., D.R. Boggs, B.J. Torok, and S.S. Boggs. 1976. Reduced blood and marrow neutrophils and granulocytic colony-forming cells in Sl/Sld mice. *Proc. Soc. Exp. Biol. Med.* 152:398–402.
16. Nocka, K., J.C. Tan, E. Chiu, T.Y. Chu, P. Ray, P. Traktman, and P. Besmer. 1990. Molecular bases of dominant negative and loss of function mutations at the murine c-*kit*/white spotting locus: W37, Wv, W41 and W. *EMBO J.* 9:1805–1813.
17. Berrozpe, G., I. Timokhina, S. Yukl, Y. Tajima, M. Ono, A.D. Zelenetz, and P. Besmer. 1999. The W(sh), W(57), and Ph Kit expression mutations define tissue-specific control elements located between -23 and -154 kb upstream of *Kit*. *Blood.* 94:2658–2666.
18. Berrozpe, G., V. Agosti, C. Tucker, C. Blanpain, K. Manova, and P. Besmer. 2006. A distant upstream locus control region is critical for expression of the Kit receptor gene in mast cells. *Mol. Cell. Biol.* 26:5850–5860.
19. Lyon, M.F., and P.H. Glenister. 1982. A new allele sash (W^{sh}) at the W-locus and a spontaneous recessive lethal in mice. *Genet. Res.* 39: 315–322.
20. Duttlinger, R., K. Manova, T.Y. Chu, C. Gyssler, A.D. Zelenetz, R.F. Bachvarova, and P. Besmer. 1993. W-sash affects positive and negative elements controlling c-*kit* expression: ectopic c-*kit* expression at sites of kit-ligand expression affects melanogenesis. *Development.* 118:705–717.
21. Duttlinger, R., K. Manova, G. Berrozpe, T.-Y. Chu, V. DeLeon, I. Timokhina, R.S.K. Chaganti, A.D. Zelenetz, R.F. Bachvarova, and P. Besmer. 1995. The W^{sh} and *Ph* mutations affect the c-*kit* expression profile: c-*kit* misexpression in embryogenesis impairs melanogenesis in W^{sh} and *Ph* mutant mice. *Proc. Natl. Acad. Sci. USA.* 92:3754–3758.
22. Chen, M., B.K. Lam, Y. Kanaoka, P.A. Nigrovic, L.P. Audoly, K.F. Austen, and D.M. Lee. 2006. Neutrophil-derived leukotriene B4 is required for inflammatory arthritis. *J. Exp. Med.* 203:837–842.
23. Kim, N.D., R.C. Chou, E. Seung, A.M. Tager, and A.D. Luster. 2006. A unique requirement for the leukotriene B4 receptor BLT1 for neutrophil recruitment in inflammatory arthritis. *J. Exp. Med.* 203:829–835.
24. Li, Y., A. Karlin, J.D. Loike, and S.C. Silverstein. 2004. Determination of the critical concentration of neutrophils required to block bacterial growth in tissues. *J. Exp. Med.* 200:613–622.
25. Mullaly, S.C., and P. Kubes. 2006. The role of TLR2 in vivo following challenge with *Staphylococcus aureus* and prototypic ligands. *J. Immunol.* 177:8154–8163.
26. Shelley, O., T. Murphy, J.A. Lederer, J.A. Mannick, and M.L. Rodrick. 2003. Mast cells and resistance to peritoneal sepsis after burn injury. *Shock.* 19:513–518.
27. Mallen-St Clair, J., C.T. Pham, S.A. Villalta, G.H. Caughey, and P.J. Wolters. 2004. Mast cell dipeptidyl peptidase I mediates survival from sepsis. *J. Clin. Invest.* 113:628–634.
28. Terato, K., K.A. Hasty, R.A. Reife, M.A. Cremer, A.H. Kang, and J.M. Stuart. 1992. Induction of arthritis with monoclonal antibodies to collagen. *J. Immunol.* 148:2103–2108.
29. Maekawa, A., K.F. Austen, and Y. Kanaoka. 2002. Targeted gene disruption reveals the role of cysteinyl leukotriene 1 receptor in the enhanced vascular permeability of mice undergoing acute inflammatory responses. *J. Biol. Chem.* 277:20820–20824.