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Basophils and mast cells play critical roles for leukocyte recruitment in IgE-mediated cutaneous reverse passive Arthus reaction

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ABSTRACT

Background: The pathogenic role of IgE has been implicated in a variety of allergic and inflammatory diseases. We have previously established an IgE-mediated cutaneous reverse passive Arthus model in which eosinophil infiltration is a prominent feature. This uniquely provides a model of type III hypersensitivity in which Fc classes of Ig that forms immune complex differentially determine the disease manifestation.

Objective: To investigate the mechanisms of how mast cells and basophils regulate this IgE-mediated Arthus reaction.

Methods: IgE-mediated cutaneous reverse passive Arthus reaction was induced in wild-type C57BL/6 or WBB6F1-+/+ mice and mast-cell-deficient WBB6F1-W/W^v mice by intradermal injection of IgE anti-trinitrophenyl antibodies followed immediately by intravenous administration of trinitrophenyl bovine serum albumin. Basophils were depleted in vivo using anti-CD200R3 monoclonal antibody prior to the IC challenge.

Results: Hemorrhage and infiltration of eosinophils, neutrophils, and basophils were significantly reduced but were not completely abrogated in WBB6F1-W/W^v mice compared with those in wild-type WBB6F1-+/+ mice. Wild-type C57BL/6 mice treated by basophil-depleting mAb also showed significantly decreased hemorrhage and inflammatory cell infiltration, especially that of eosinophils, compared with control mice. Furthermore, basophil depletion in WBB6F1-W/W^v mice led to nearly complete inhibition of eosinophil recruitment. By contrast, basophil depletion did not further decrease neutrophil infiltration in WBB6F1-W/W^v mice.

Conclusion: While mast cells play a central role, basophils also have an important function, especially for eosinophil recruitment, in IgE-mediated cutaneous reverse passive Arthus reaction.

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1. Introduction

Mast cells are implicated in the pathogenesis of allergic diseases by their ability to be activated through both FcεRI-bound antigen-specific IgE and FcγRIII-bound antigen-specific IgG1 [1–6]. Activated mast cells release various proinflammatory mediators, including histamine, eicosanoids and a number of cytokines. Among the

cytokines, tumor necrosis factor-α upregulates endothelial and epithelial adhesion molecules to enhance neutrophil adhesion to endothelial cells, while interleukin (IL)-3, granulocyte macrophage colony-stimulating factor, and IL-5, produced by mast cells, are critical for eosinophil development and survival. Accumulating evidences indicate that mast cells are not only involved in allergic disorders, such as anaphylaxis and asthma, but also actively participate in diverse immune responses and contribute to the pathology of various disorders [1–3,7,8].

Basophils share many features with mast cells, including expression of FcεR1, metachromatic staining, and release of histamine after activation [8,9]. However, mast cells and basophils constitute distinct lineages having many unique features. In contrast to mast cells, basophils are circulating granulocytes that

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typically mature in the bone marrow, circulate in the blood as mature cells, and can be recruited into tissues at sites of immunological or inflammatory responses [8,9]. Recent studies have revealed a variety of functions of basophils. Basophils secrete Th2 (Th2) cytokines, such as IL-4 and IL-13, in both humans and mice [8,10–12], suggesting that the basophil is a key regulator in conditioning the immune response to Th2 reaction. Basophils have also been demonstrated to play an essential role in the development of IgE-mediated chronic allergic inflammation in the skin, independently of mast cells [13,14]. In addition, basophils are a main contributor for IgG-mediated anaphylaxis through release of platelet-activating factor (PAF) instead of histamine [15,16].

Deposition of the antigen (Ag) and antibody (Ab) complexes in tissues is one of the hallmarks of immune diseases. The Arthus reaction, classified in type III hypersensitivity, is the prototypic *in vivo* model for immune complex (IC)-mediated acute inflammatory tissue injury [17]. In the reverse passive Arthus reaction, an excess of Ab is injected into the skin of animals previously subjected to intravenous (i.v.) injection with the corresponding Ag. The early animal studies led to general agreement that complement activation by ICs is the key initiating step and demonstrated a requirement for neutrophils and mast cells for propagation of the inflammatory cascade [18–20]. However, more recent studies have suggested that IC-mediated inflammation in the reverse passive Arthus reaction is initiated via Fcγ receptor-triggered pathways. In addition, it has been reported that, in mice deficient in complement protein C3, C4, or C5, the reverse passive Arthus reaction develops normally [21]. We have previously established an IgE-mediated cutaneous reverse passive Arthus model in mice carrying IgE anti-TNP Abs [22]. Different from IgG-mediated immune IC-complex challenge, which is neutrophil dominant, IgE-mediated Arthus reaction induced prominent eosinophil infiltration in addition to other inflammatory cells. This model demonstrates that Fc classes of Ig that forms IC critically determine the disease manifestation. However, the mechanisms of the recruitment of eosinophils in IgE-mediated cutaneous reverse passive Arthus reaction in mouse as yet remain to be clarified. In this study, we demonstrated that both mast cells and basophils are important mediators in IgE-mediated cutaneous reverse passive Arthus reaction and that basophils play a considerable role especially in infiltration of eosinophils.

2. Materials and methods

2.1. Mice

C57BL/6, WBB6F1-+/+ and WBB6F1-W/W^v mice were obtained from Japan SLC Inc (Hamamatsu, Japan). All mice were healthy, fertile, and did not display evidence of infection or disease. All mice used for experiments were 10–14 weeks old. All mice were housed in a pathogen-free barrier facility and screened regularly for pathogens. All the experiments in this study were performed according to the Guidelines for Animal Use and Experimentation, as set out by Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University.

2.2. Cutaneous reverse passive Arthus reaction

To induce cutaneous reverse passive Arthus reaction [23–25], mice were anesthetized by inhalation of diethyl ether, shaved on their abdominal skin, and wiped with 70% ethanol. Mouse IgE anti-trinitrophenyl (TNP) Abs (60 μg/30 μl; BD Biosciences, San Diego, CA) were injected once intradermally into the abdominal skin of the mice using a 29-gauge needle, followed immediately by i.v. injection of 2,4,6-trinitrophenyl bovine serum albumin (TNP-BSA, 800 μg/400 μl; LSL, Japan) in PBS via the tail vein. The intradermal

injection of mouse IgE anti-TNP Abs (60 μg/30 μl) followed by i.v. installation of BSA in PBS served as a control. For basophil depletion, Ba103 mAb specific for CD200R3 (30 μg per mouse) was given an intravenous injection 1 day before the IC challenge [13]. Rat IgG (30 μg per mouse; Sigma–Aldrich, St. Louis, MO) served as a control.

The amount of hemorrhage was assessed 24 h after IC challenge. Mice were killed, and the skin containing the injection site was removed at the level of the fascia above the skeletal muscle and was reversed. The diameters of purpuric spots and bleeding vessels on the fascia side of the injection site were measured using Adobe Photoshop 5.0 software. The diameters of the major and minor axes of the purpuric spots were averaged. Then, the ratio of purpuric spot diameter/bleeding vessel diameter was evaluated [22].

2.3. Flow cytometry analysis

R-phycoerythrin (PE)-conjugated anti-FcεRIα (MAR-1) and fluorescein isothiocyanate (FITC)-conjugated CD49b (HMα2) mAbs were purchased from eBioscience (San Diego, CA). Blood (50 μl) was obtained via retro orbital route before and 1 day after Ba103 administration. The stained cells were analyzed with a FACSCalibur (BD Biosciences).

2.4. Histological examination and immunohistochemical staining

Tissues were harvested before and 24 h after IC challenge using a disposable, sterile 6-mm punch biopsy blade (Maruho, Osaka, Japan) and assessed for tissue damage and number of infiltrating eosinophils, neutrophils, and mast cells. Tissues were cut into halves, fixed in 3.5% paraformaldehyde, and then embedded in paraffin. Six-micrometer sections were stained using hematoxylin and eosin (H&E) for inflammatory cell evaluation and toluidine blue for mast cell evaluation. Hansel staining was used for eosinophil evaluation [26]. To identify neutrophils and basophils, polyclonal anti-myeloperoxidase (anti-MPO; Thermo Scientific, Fremont, CA) Abs and anti-mast cell serine protease-8 (MCP-8) mAb (BioLegend; San Diego, CA), respectively, were used on paraffin-embedded sections by the following procedure [27].

All extravascular cells were counted from the infection site in five serial skin sections. The numbers of eosinophils and neutrophils were averaged in eight power microscopic fields (HPFs, 0.07 mm²) and mast cells and basophils were counted in the entire section. Each section was examined independently by three investigators in a blinded fashion, and the mean was used for analysis.

2.5. Statistical analysis

The student *t*-test was used for determining the level of significance of differences in sample means and Bonferroni's test was used for multiple comparisons. A *P* value <0.05 was considered significant. All data are shown as means ± SEM.

3. Results

3.1. Mast-cell deficient mice were able to mount an IgE-mediated cutaneous reverse passive Arthus reaction

It is widely accepted that the mast cell is the major source of hypersensitivity [2,3,5], while the role of mast cells in the IgE-mediated cutaneous reverse passive Arthus reaction has not been investigated. To assess whether deficiency of mast cells can abolish IgE-mediated cutaneous reverse passive Arthus reaction, WBB6F1-+/+ and WBB6F1-W/W^v mice littermates were injected with IgE anti-TNP Abs, followed immediately by i.v. installation of TNP-BSA,

as reported previously [22]. Hemorrhage was evaluated 24 h after IC challenge. Since the size of hemorrhage was associated with the diameter of the blood vessels, the ratio of the purpuric spot/bleeding vessel diameters was evaluated for the analysis. Hemorrhage was

observed in both WBB6F1-+/+ and WBB6F1-W/W^v mice (Fig. 1a); however, WBB6F1-W/W^v mice showed smaller hemorrhage (Fig. 1a) and significantly decreased diameter ratio compared with WBB6F1-+/+ mice (Fig. 1b).

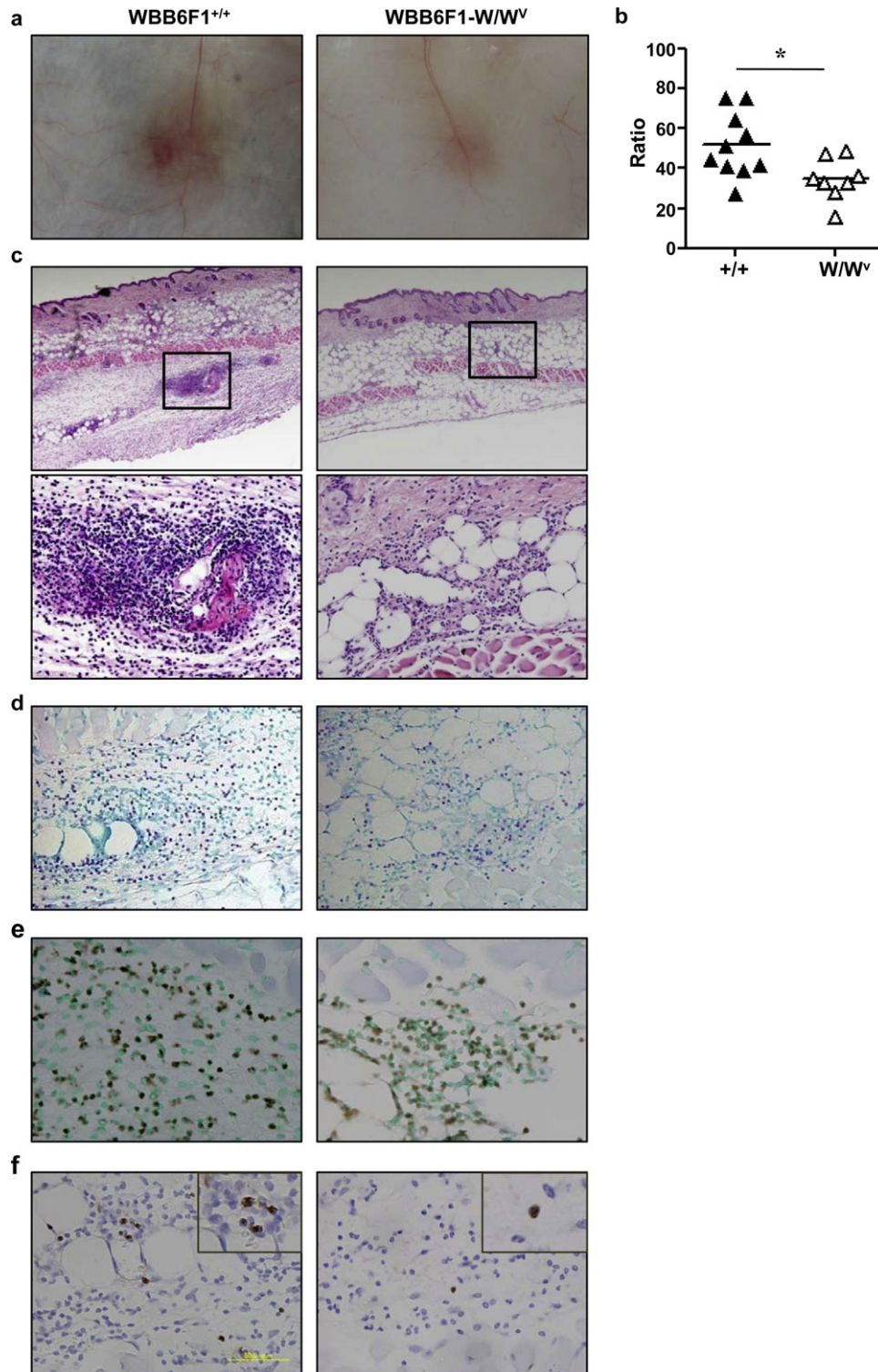


Fig. 1. IgE-mediated cutaneous reverse passive Arthus reaction in mast cell-deficient mice. WBB6F1-+/+ and WBB6F1-W/W^v mice were injected intradermally with IgE anti-TNP Abs, followed immediately by intravenous installation of TNP-BSA or BSA as control. Hemorrhage was evaluated 24 h after IC challenge. Representative pictures are shown in (a). The diameter ratio, indicating the mean diameters of purpuric spots divided by the diameter of bleeding vessels, was determined in WBB6F1-+/+ and WBB6F1-W/W^v mice (b). Horizontal lines indicate mean values for each group of mice. Histological tissue sections showed infiltration of inflammatory cells in skin lesions of WBB6F1-+/+ and WBB6F1-W/W^v mice. H&E staining demonstrated a damaged vessel with prominent inflammation and leukocytoclastic vasculitis with eosinophil infiltration in WBB6F1-+/+ and WBB6F1-W/W^v mice (c). Original magnifications, $\times 40$ (c, top) and $\times 200$ (c, bottom). Eosinophils (d), neutrophils (e) and basophils (f) were detected by Hansel, anti-MPO and anti-MCP-8 staining, respectively. Original magnifications, $\times 200$ (d) and $\times 400$ (e and f).

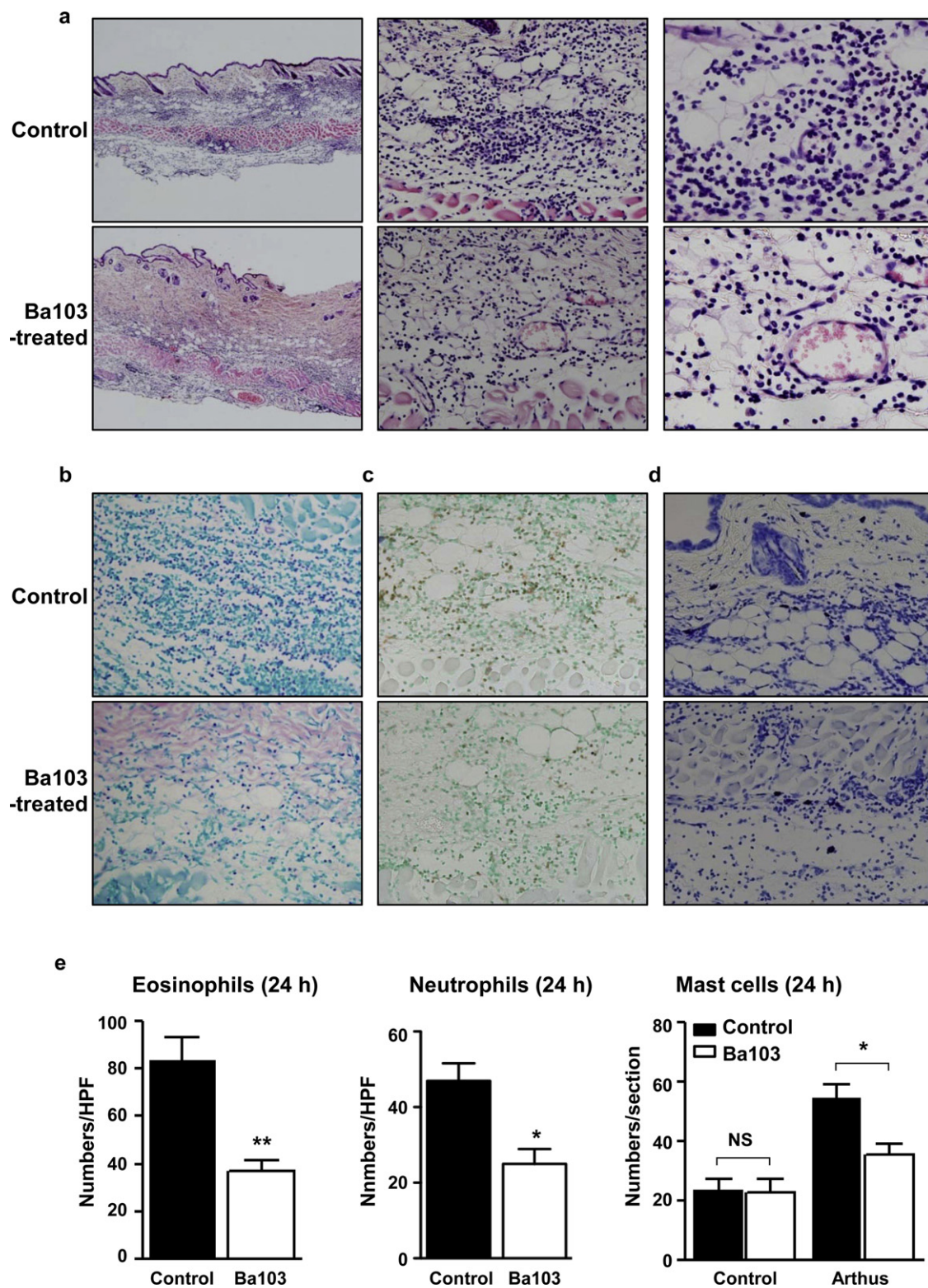


Fig. 2. IgE-mediated cutaneous reverse passive Arthus reaction in basophil-depleted mice. IgE-mediated cutaneous reverse passive Arthus reaction established in C57BL/6 mice, as described in Fig. 1. Mice were treated with 30 μ g Ba103 mAb by intravenous injection 1 day before the antigen challenge. H&E staining demonstrated a damaged vessel with prominent inflammation and leukocytoclastic vasculitis with eosinophil infiltration at 24 h after IC challenge in Ba103-treated and control wild type mice (a). Original magnifications, left $\times 40$; middle $\times 200$; right $\times 400$ (a). Eosinophil (b), neutrophil (c) and mast cell (d) accumulation in skin lesions at 24 h after IC challenge. Eosinophils were detected by Hansel staining (b); neutrophils by anti-MPO Ab staining (c); and mast cells by metachromatic staining of granules in toluidine blue-staining sections (d). Original magnification $\times 200$ (b–d). The numbers of eosinophils (a), neutrophils (b) and mast cells (c) in the skin section from Ba103-treated or control wild type mice were determined (bottom graphs). All data are expressed as the mean $\pm$ SEM of in each group and are representative of three repeated experiments. Significant differences between sample means are indicated; * $p < 0.05$; ** $p < 0.01$.

Eosinophils and neutrophils dominate the infiltrated inflammatory cells in the IgE-mediated cutaneous reverse passive Arthus reaction [22]. Histopathological analysis showed markedly decreased infiltration of inflammatory cells in the WBB6F1-W/W^v mice compared with that in the WBB6F1-+/+ mice (Fig. 1c). Nonetheless, substantial inflammatory cells were still observed in WBB6F1-W/W^v mice, including eosinophils (Fig. 1d) and neutrophils (Fig. 1e and supplementary Fig. 1a) as were revealed in skin tissue sections subjected to Hansel staining and anti-MPO Ab staining, respectively. Thus, while mast cells play a critical role in initiating the IgE-mediated cutaneous reverse passive Arthus reaction, other components also participate in this response.

Basophil infiltration was also assessed in skin tissue section by staining with anti-MCP-8 mAb. While basophil infiltration in the skin was observed in both WBB6F1-+/+ and WBB6F1-W/W^v mice after IC challenge (Fig. 1f and supplementary Fig. 1b), WBB6F1-W/W^v mice exhibited a significantly lower number of basophils than in WBB6F1-+/+ mice at 24 h (8.3 ± 2.7 /section in WBB6F1-W/W^v mice vs. 69.0 ± 8.7 /section in WBB6F1-+/+ mice, $p < 0.05$; Fig. 1f). These results suggest that basophil recruitment is crucially but not completely dependent on mast cells.

3.2. In vivo depletion of basophils efficiently ameliorated inflammatory reaction

Basophils also express FcεRI [1,28]. To study the role of basophils in IgE-mediated cutaneous reverse passive Arthus reaction, we treated C57BL/6 mice with basophil-depleting mAb (Ba103) via i.v. injection. As was reported previously [13], the in vivo administration of Ba103 induced a drastic reduction in the number of peripheral basophils identified as CD49b⁺FcεRI⁺ cells (supplementary Fig. 2a and b), but did not influence the numbers of

other circulating leukocytes including neutrophils and eosinophils or skin-residing cells (data not shown).

Leukocyte infiltration was assessed in skin tissue sections after 24 h of IC formation. While histopathology revealed the destruction of blood vessels with inflammatory cell infiltration and extravasation of red blood cells in control C57BL/6 mice that were not pretreated with Ba103 as was observed in WBB6F1-+/+ mice, the administration of Ba103 mAb substantially ameliorated inflammatory reaction (Fig. 2). In particular, depletion of basophils significantly decreased eosinophil invasion, which was $74.5 \pm 6.3\%$ lower than that in control mice (Fig. 2b and e). Neutrophil infiltration was also significantly decreased by Ba103 treatment, although the inhibitory effect ($46.8 \pm 3.7\%$ decrease) was smaller than that on eosinophils (Fig. 2c and e). Additionally, the number of mast cells was also reduced by Ba103 treatment compared with that in control mice (Fig. 2d and e). Thus, the blockade of basophils results in diminution of inflammatory reaction after IC challenges in vivo. Collectively, basophils also play a substantial role in the IgE-mediated cutaneous reverse passive Arthus reaction.

3.3. Both mast cells and basophils are responsible for induction of IgE-mediated cutaneous reverse passive Arthus reaction

The results presented above suggested that not only mast cells but also basophils contributed to IgE-mediated cutaneous reverse passive Arthus reaction. However, Ba103 treatment might affect mast cell functions as well. Therefore, to elucidate the role of basophils in this reaction, we next examined WBB6F1-+/+ and WBB6F1-W/W^v mice treated with or without Ba103 1 day before induction of IgE-mediated Arthus reaction. Ba103-treated WBB6F1-+/+ mice exhibited a reduction of hemorrhage ratio that was significantly lower than that of control WBB6F1-+/+ mice

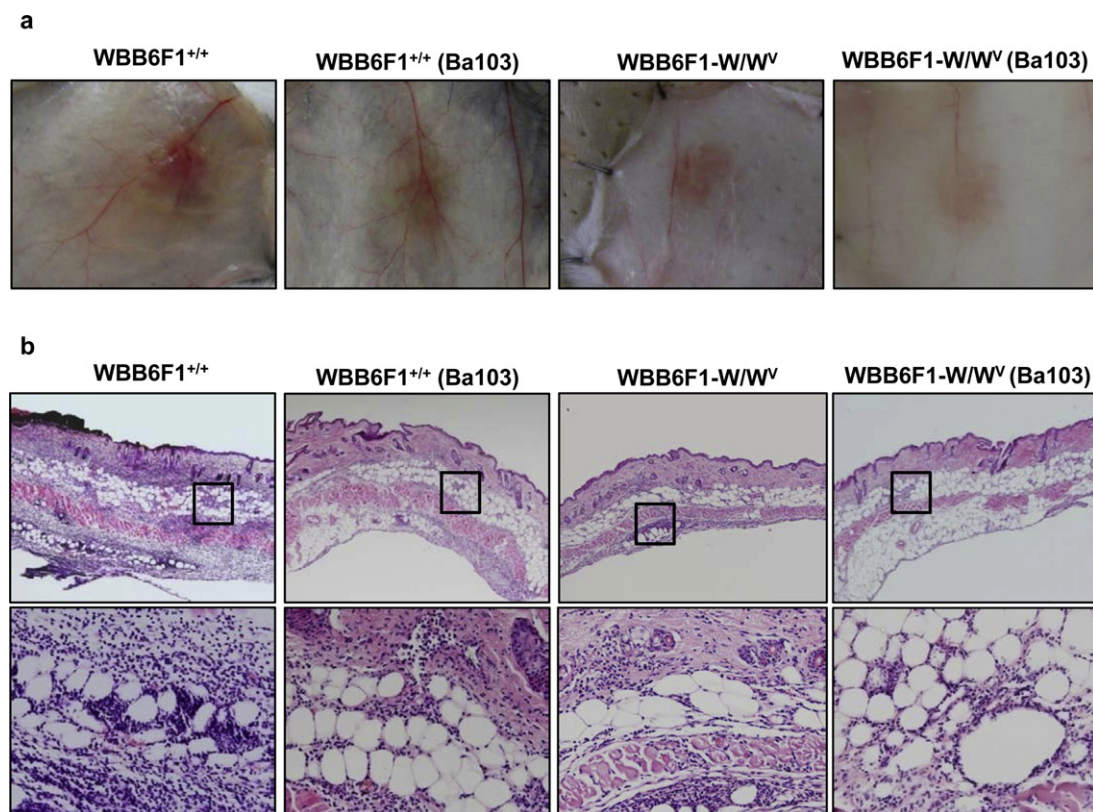


Fig. 3. Mast cell deficiency and basophil depletion in IgE-mediated cutaneous reverse passive Arthus reaction. Histological tissue sections showed hemorrhage and recruitment of inflammatory cells in the skin from WBB6F1-W/W^v mice and WBB6F1-+/+ mice treated with or without Ba103 Ab. Hemorrhage was evaluated 24 h after IC challenge (a). Inflammation reaction was revealed by H&E staining (b). Original magnifications, top $\times 40$; bottom $\times 200$ (b).

(Fig. 3a). WBB6F1-W/W^v mice showed further reduction of hemorrhage ratio relative to WBB6F1-+/+ mice (Figs. 1a and 3a). The hemorrhage size (i.e. diameter ratio) was slightly inhibited in basophil-depleted WBB6F1-W/W^v mice compared with control WBB6F1-W/W^v mice, although the difference was not statistically significant (Fig. 3a).

Infiltrating cells were also assessed in skin tissue sections by H&E staining after 24 h of IC formation. Ba103 administration resulted in greater inhibition of infiltration of inflammatory cells in WBB6F1-+/+ and WBB6F1-W/W^v mice (Fig. 3b) than that in

corresponding control groups. Furthermore, basophil blockade in mast cell-deficient mice nearly completely inhibited IgE-mediated cutaneous reverse passive Arthus reaction (Fig. 3b).

3.4. The basophil is the major initiator of eosinophil recruitment, but is dispensable for neutrophil recruitment, in mast cell deficient mice

Following basophil depletion, infiltrating eosinophil numbers were significantly reduced in both WBB6F1-+/+ and WBB6F1-W/W^v mice compared with those in control mice (Fig. 4a and b). In

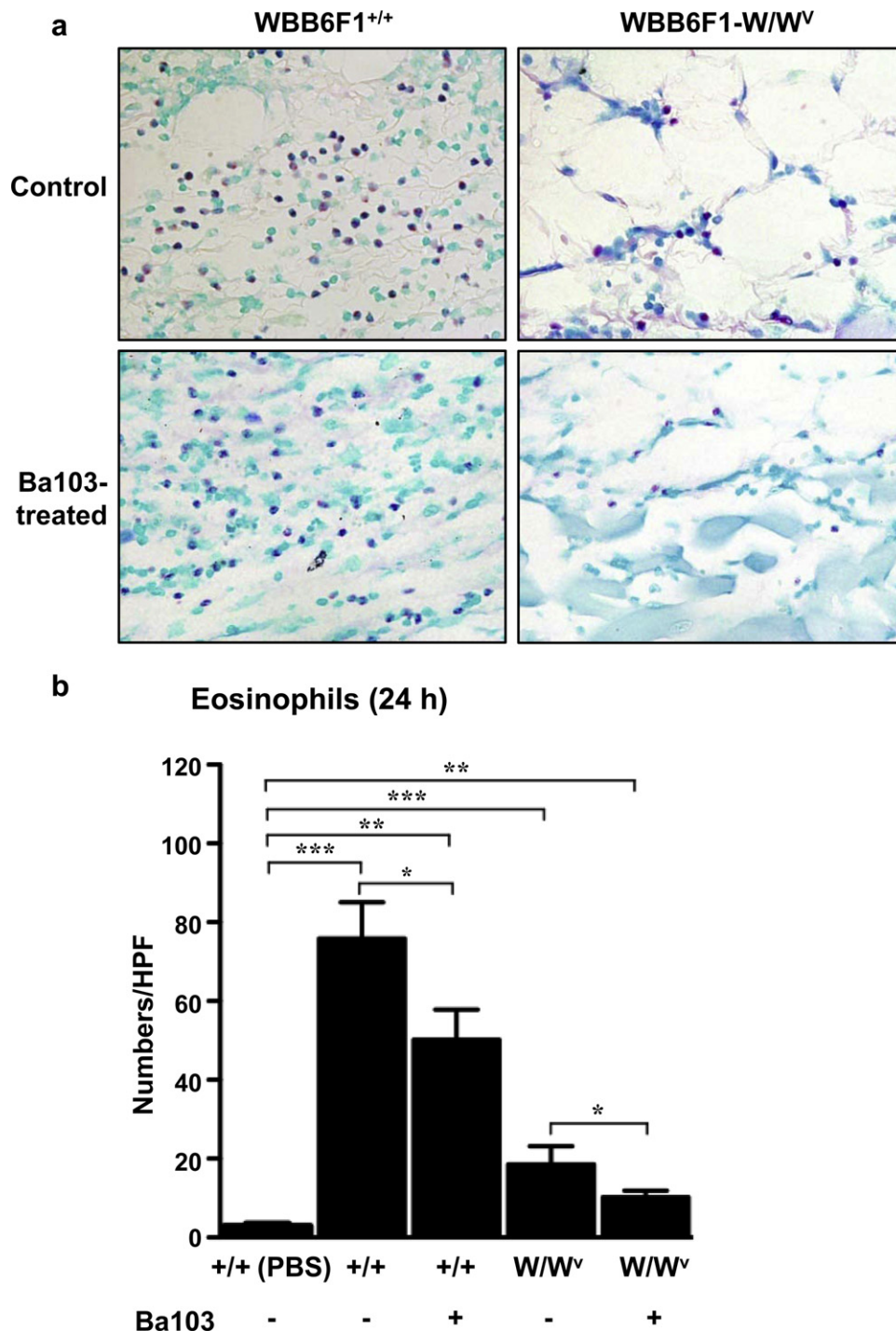


Fig. 4. Eosinophil infiltration in IgE-mediated cutaneous reverse passive Arthus reaction in mast cell-deficient mice and basophil-depleted mice. Histological tissue sections showed eosinophil accumulation in the skin of WBB6F1-+/+ and WBB6F1-W/W^v mice treated with or without Ba103. All data are expressed as the mean $\pm$ SEM and were obtained from ≥ 3 mice in each group. Original magnifications, left $\times 200$; right $\times 400$ (a). Significant differences between sample means are indicated; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Similar results were obtained in at least three independent experiments.

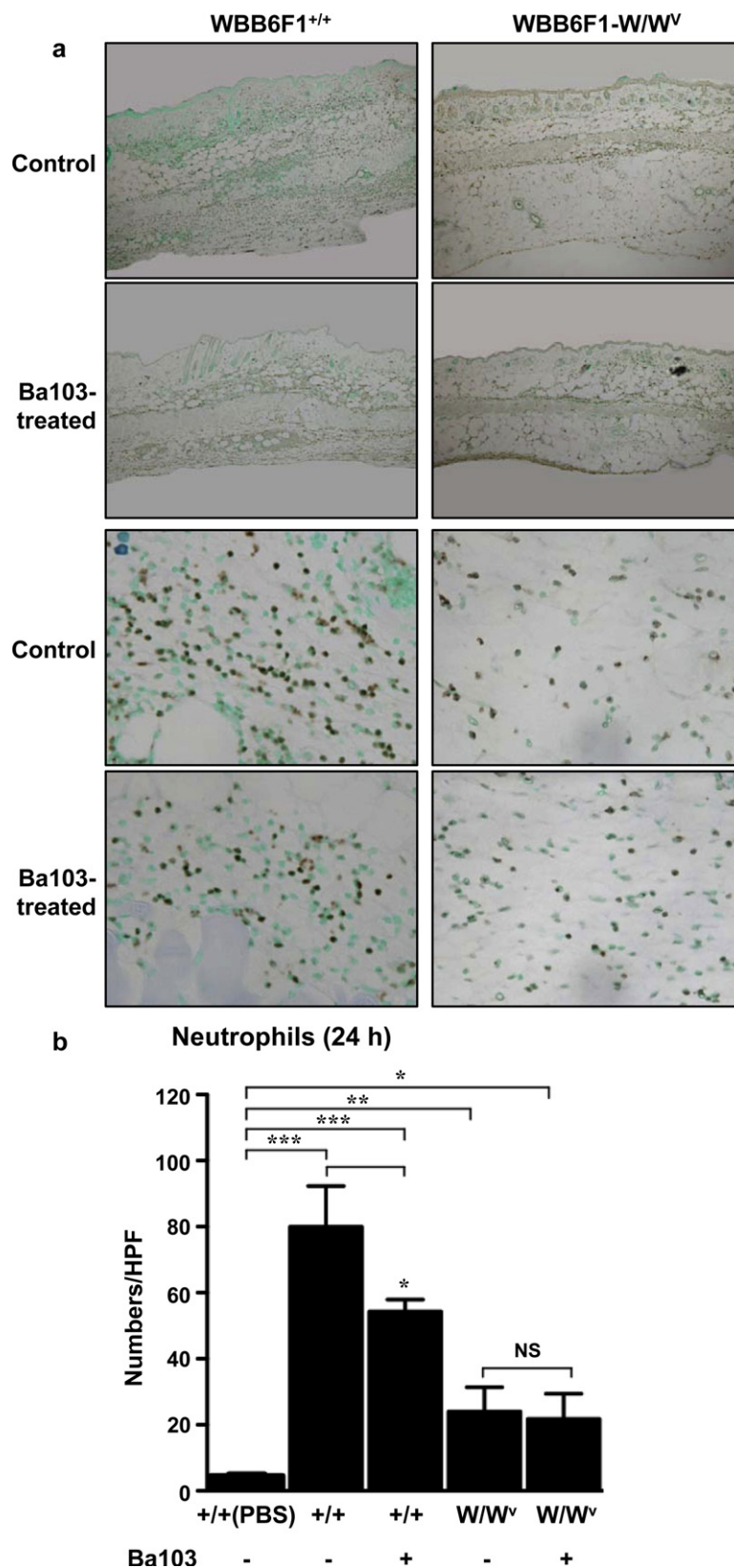


Fig. 5. Neutrophil infiltration in IgE-mediated cutaneous reverse passive Arthus reaction in mast-cell-deficient mice and basophil-depleted mice. Histological tissue sections showed neutrophil accumulation in the skin of WBB6F1^{+/+} and WBB6F1-W/W^v mice treated with or without Ba103 Ab. All values represent the mean $\pm$ SEM. Results were obtained from ≥ 3 mice in each group. Original magnifications, low $\times 40$; middle $\times 200$; high $\times 400$ (a). Significant differences between sample means are indicated; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The results represent ≥ 2 independent experiments with three mice in each group.

particular, eosinophils were almost completely inhibited by basophil depletion in WBB6F1-W/W^v mice (Fig. 4b). Thus, in addition to mast cells, basophils likely play an essential role in inducing infiltrating eosinophils in IgE-mediated cutaneous reverse passive Arthus reaction.

The numbers of neutrophils that extravasate from blood vessels were assessed in the skin tissues by anti-MPO Ab staining. In the WBB6F1-+/+ mice, Ba103-treated mice showed significantly reduced neutrophil numbers (Fig. 5a and b). However, there were no significant differences between Ba103 treated and control group in the WBB6F1-W/W^v mice (Fig. 5b). Thus, mast cells are likely to have a principal role for neutrophil recruitment.

4. Discussion

Prominent eosinophil infiltration is a hallmark of IgE-mediated Arthus reaction compared with IgG-mediated Arthus reaction [22]. In the current study, we demonstrated that mast cells and basophils are responsible for the specific pattern of eosinophil-dominant recruitment in IgE-mediated cutaneous reverse passive Arthus reaction. Mast cell-deficient WBB6F1-W/W^v mice exhibited significantly smaller hemorrhage (Fig. 1a and b) and reduced numbers of infiltrating eosinophils, neutrophils and basophils (Fig. 1c–f), confirming that the mast cell is the major contributor to the Arthus reaction. Nonetheless, substantial inflammatory reaction was still induced in the absence of mast cells. Basophil depletion also significantly reduced the reaction in the presence or absence of mast cells. Especially, basophil depletion significantly decreased tissue eosinophilia both in wild-type and mast cell-deficient mice, demonstrating their important role in eosinophil infiltration in the skin. The results demonstrated that, while mast cells are the dominant regulators, basophils also play a significant role in eliciting IgE-mediated cutaneous reverse passive Arthus reaction.

While basophils have long been considered as “immature circulating mast cells”, recent studies have revealed that they represent a distinct lineage and play non-redundant roles from mast cells in a variety of immune responses. In immediate-type allergy, Tsujimura et al. demonstrated that basophils are dispensable for IgE-mediated systemic anaphylaxis [15]. Instead, basophils are responsible for IgG-mediated systemic anaphylaxis, which is consistent with previous studies showing that mice deficient in mast cells, IgE, or FcRI still develop systemic anaphylaxis [4,5,28–31]. Additionally, neutrophils express FcγRs and have been demonstrated to induce anaphylaxis [32]. By contrast, in delayed-type allergy, basophils play an essential role in the development of IgE-mediated chronic allergic reactions in the skin characterized by significant eosinophil infiltration by functioning as initiator cells in mice [14,16]. In the present study, depletion of basophils via tail vein injection of Ba103 significantly decreased cellular infiltration, especially that of eosinophils, compared with the level in control wild type mice (Figs. 2 and 3). Furthermore, Ba103 treatment in WBBF1-W/W^v mice resulted in nearly complete abolishment of eosinophil infiltration (Fig. 4a and b). Thus, our results have demonstrated that basophils have a distinct role in eosinophil recruitment in this IgE-mediated reaction. By contrast, the number of infiltrated neutrophils did not differ between Ba103-treated and control groups in WBBF1-W/W^v mice (Fig. 5a and b). While this may suggest a relatively minor role of basophils in neutrophil recruitment, basophil depletion by Ba103 significantly reduced the numbers of infiltrated neutrophils in wild-type C57BL/6 mice and WBBF1-+/+ mice (Figs. 2e and 5b). Since mast cells contribute to basophil recruitment to the skin in this IgE-mediated reaction, basophils, once recruited to the site of inflammation, then play an important role in neutrophil accumulation as well.

Despite the absence of both mast cells and basophils, we still observed some neutrophils in the skin lesion. In IgG-mediated Arthus reaction, chemotaxis of complement C3a or C5a have been demonstrated [33,34]. Coxon et al. demonstrated that IC bound to the endothelium supports the capture of neutrophils via interaction with FcγRIII [35]. While IgE is not considered to bind with complement components, other receptors may be involved in an analogous mechanism to recruit neutrophils. FcεRI is expressed not only in mast cells and basophils but also in dendritic cells. Alternatively, in the absence of mast cells and basophils, CD23-bearing cells may play a role in the reaction.

In this IgE-mediated Arthus reaction model, IgE is initially delivered into skin tissue where mast cells, but not basophils, reside. Immune complexes are formed locally in the lesion after Ag challenge and activate tissue-resident mast cells. Chemical mediators released from mast cells are likely to attract basophils, neutrophils and eosinophils [36,37]. Consistent with this, the numbers of eosinophils, neutrophils and basophils infiltration were significantly decreased in mast cell-deficient WBB6F1-W/W^v mice (Fig. 1). Basophils recruitment may amplify the inflammatory reaction by degranulation, release of cytokines, chemokines and other chemokine mediators. Moreover, basophils have been shown to secrete prostaglandin D2 (PGD2) in response to IgE and Ag, but not to IgG and Ag. PDG2 recruits and activates eosinophils as well as basophils and Th2 cells. Eosinophils have also been reported to produce PDG2. Thus, PDG2 may provide autocrine activation signal for both eosinophils and basophils, which likely plays additional role in the inflammatory site [38,39]. In the current study, depletion of basophils significantly decreased the inflammatory reaction.

A recent study revealed that, in human skin diseases, basophils are generally detected in skin lesions where eosinophils are present, including bullous pemphigoid and eosinophilic pustular folliculitis [38,40]. Furthermore, Otsuka et al. reported that basophils infiltration was observed in most cases of eosinophilic pustular folliculitis but not in neutrophilic folliculitis [39]. As for vasculitis, a number of basophils are detected in skin lesions of Henoch-Schönlein purpura [40], a prototypic cutaneous vasculitis, although the disease is mediated by IgA but not IgE. Nonetheless, this may indicate that basophils have an important role in cutaneous vasculitis in humans as well.

In summary, our data suggest that mast cells and basophils synergistically participate in IgE-mediated reverse passive cutaneous Arthus reaction. Therapy targeting basophils may be of potential interest in treating related disorders.

Acknowledgments

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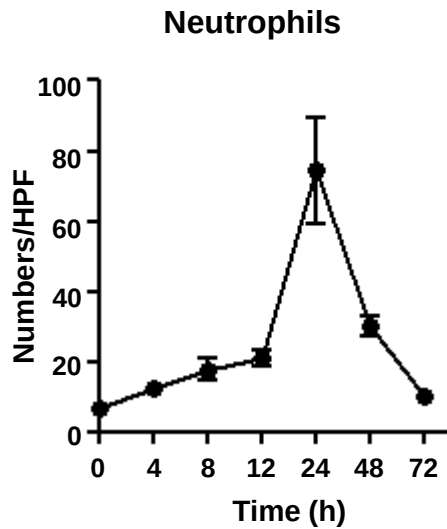
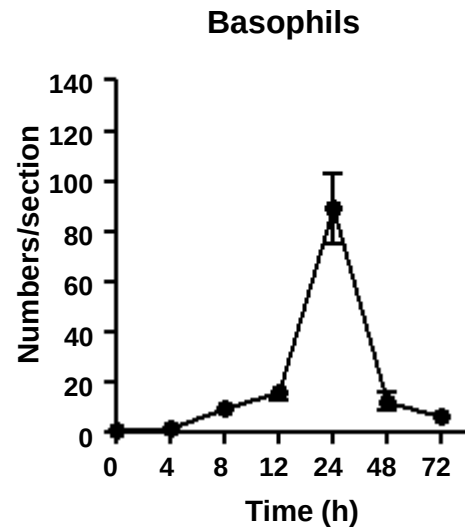
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jdermsci.2012.06.005>.

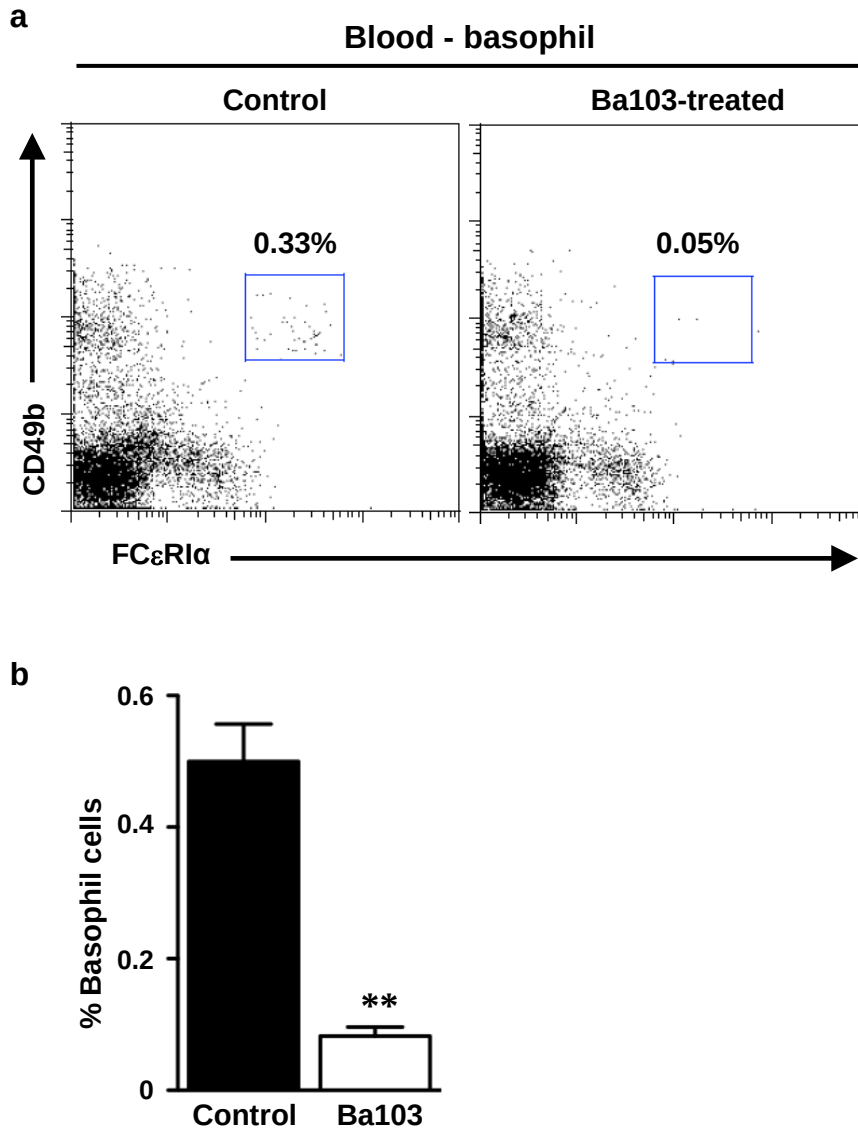
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a**b**

Supplementary Fig. 1 Time course of neutrophil (a) and basophil (b) infiltration in the skin in IgE-mediated Arthus reaction. Wild type mice were injected with IgE anti-TNP Ab intradermally, followed by i.v. TNP-BSA injection. The numbers of neutrophils and basophils were determined before and after IC challenge at 4, 8, 12, 24, 48, and 72 h, respectively. All values represent the mean $\pm$ SEM.



Supplementary Fig. 2 Depletion of basophils by Ba103 mAb administration. C57BL/6 mice were treated with an intravenous injection of 30 μ g Ba103 or control antibody. Peripheral blood cells were isolated 24 h later and stained for FcεR1α and CD49b to identify basophils. (a and b) The numbers showed the percentage of basophils in control or Ba103-treated group. Data are expressed as the mean $\pm$ SEM of in each group and are representative of 3 repeated experiments. Significant differences between sample means are indicated; ** $p < 0.01$.