Basophils regulate the recruitment of eosinophils in a murine model of irritant contact dermatitis

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Background: Although eosinophils have been detected in several human skin diseases in the vicinity of basophils, how eosinophils infiltrate the skin and the role of eosinophils in the development of skin inflammation have yet to be examined.

Objective: Using murine irritant contact dermatitis (ICD) as a model, we sought to clarify the roles of eosinophils in ICD and the underlying mechanism of eosinophil infiltration of the skin.

Methods: We induced croton oil–induced ICD in eosinophil-deficient ΔdblGATA mice with or without a reactive oxygen species (ROS) inhibitor. We performed cocultivation with fibroblasts and bone marrow–derived basophils and evaluated eosinophil migration using a chemotaxis assay.

Results: ICD responses were significantly attenuated in the absence of eosinophils or by treatment with the ROS inhibitor. ROS was produced abundantly by eosinophils, and both basophils and eosinophils were detected in human and murine ICD skin lesions. In coculture experiments, basophils attracted eosinophils, especially in the presence of fibroblasts. Moreover, basophils produced IL-4 and TNF-α in contact with fibroblasts and promoted the expression of eotaxin/CCL11 from fibroblasts *in vitro*.

Conclusion: Eosinophils mediated the development of murine ICD, possibly through ROS production. Recruitment of eosinophils into the skin was induced by basophils in cooperation with fibroblasts. Our findings introduce the novel concept that basophils promote the recruitment of eosinophils into the skin through fibroblasts in the development of skin inflammation. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Eosinophil, basophil, fibroblast, eotaxin/CCL11, RANTES/CCL5, irritant contact dermatitis, reactive oxygen species, tumor necrosis factor*

Contact dermatitis is one of the most common inflammatory skin diseases and comprises both irritant contact dermatitis (ICD) and allergic contact dermatitis.[1] ICD is more common than allergic contact dermatitis and is responsible for approximately 80% of all cases of contact dermatitis.[2] It is defined as a locally arising reaction that appears after chemical irritant exposure.[2] The chemical agents are directly responsible for cutaneous inflammation because of their inherent toxic properties, which cause tissue injury.[3,4] This inflammatory response is known to activate innate immune system cells, but the precise mechanism of ICD remains largely unknown.

Eosinophils are one of the bone marrow (BM)–derived innate immune leukocytes that normally represent less than 5% of leukocytes in the blood but are frequently detected in the connective tissues and BM.[5] Eosinophils regulate local immune and inflammatory responses, and their accumulation in the blood and tissue is associated with several inflammatory and infectious diseases.[6-8] The recruitment of activated eosinophils from the bloodstream into tissues occurs under numerous conditions and leads to the release of preformed and synthesized products, such as cytokines, chemokines, lipid mediators, cytotoxic granule proteins, and reactive oxygen species (ROS).[7,9] ROS are mainly produced by reduced nicotinamide adenine dinucleotide phosphate oxidase and lead to tissue injury at the inflamed site during allergic inflammation.[10] The differentiation, migration, and activation of eosinophils are mainly enhanced by IL-5.[11] It has been reported that the IL-5–targeted therapy can reduce airway and blood eosinophil counts and prevent asthma exacerbations[12]; however, the roles of eosinophils in the development of cutaneous immune responses remain largely unknown. It has been recently reported that basophils have been detected in patients with skin diseases, including contact dermatitis, in which eosinophils were present.[13,14]

Basophils are one of the least abundant granulocytes, representing less than 1% of peripheral blood leukocytes.[15] Their specific physiologic functions during immune responses have been ignored until recently. Basophils play key roles in the development of acute and chronic allergic responses, protective immunity against parasites, and regulation of acquired immunity, including the augmentation of humoral memory responses.[16,17]

In this study we observed the infiltration of eosinophils in human and murine ICD. Murine ICD responses were attenuated in eosinophil-deficient mice or in mice treated with an ROS inhibitor. ROS was produced by eosinophils, which were attracted by chemokines produced through interaction between basophils

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Abbreviations used

APC: Allophycocyanin
Bas TRECK: Basophil-specific enhancer-mediated, toxin receptor-mediated conditional cell knockout
BM: Bone marrow
BMBa: Bone marrow–derived basophil
BMEo: Bone marrow–derived eosinophil
CBA: Cytometric bead array
CM-H_2DCFDA: 5-(and-6)-chloromethyl-2′, 7′-dichlorodihydrofluorescein diacetate
cRPMI: Complete RPMI medium
DT: Diphtheria toxin
Flt3-L: Fms-related tyrosine kinase 3 ligand
H&E: Hematoxylin and eosin
ICD: Irritant contact dermatitis
IgE-CAI: IgE-mediated chronic allergic inflammation
MEF: Mouse embryonic fibroblast
NAC: N-acetylcysteine
PE: Phycoerythrin
RA: Rheumatoid arthritis
ROS: Reactive oxygen species
SCF: Stem cell factor
Tg: Transgenic
TSLP: Thymic stromal lymphopoietin
WT: Wild-type

and fibroblasts. Our findings might raise an important concept that the interaction between basophils and mesenchymal fibroblasts induces the development of ICD through recruitment of eosinophils.

METHODS

Mice

ΔdblGATA mice on a BALB/c background were purchased from the Jackson Laboratory (West Grove, Pa). IL-5 transgenic (Tg) mice on a BALB/c background[18] were kindly provided by Dr K. Takatsu (University of Toyama, Toyama, Japan). Basophil-specific enhancer-mediated, toxin receptor-mediated conditional cell knockout (Bas TRECK) mice on a BALB/c background were generated, as reported previously. Briefly, basophils use a specific 4-kb enhancer fragment containing the 3′ untranslated region and DNase I-hypersensitive site 4 elements to regulate *Il4* gene expression.[19] Using this system, we generated mice that express human diphtheria toxin (DT) receptor under the control of HS4.[17,20] By using these mice, basophils have been reported to play an essential role for the induction and promotion of T_H2 immunity.[17,21] C57BL/6N and BALB/c wild-type (WT) mice were purchased from Japan SLC (Shizuoka, Japan). Eight- to 10-week-old female mice were used for all the experiments and bred in specific pathogen-free facilities at Kyoto University. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Kyoto University Graduate School of Medicine (Kyoto, Japan).

Reagents, antibodies, and flow cytometry

We purchased croton oil and N-acetylcysteine (NAC) from Sigma-Aldrich (St Louis Mo). 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) was purchased from Invitrogen (Carlsbad, Calif). Recombinant murine stem cell factor (SCF), fms-related tyrosine kinase 3 ligand (Flt3-L), and IL-3 were purchased from PeproTech (Rocky Hill, NJ). Recombinant mouse IL-5 was purchased from R&D Systems (Minneapolis, Minn). Fluorescein isothiocyanate–, phycoerythrin (PE)–, PE-Cy7–, allophycocyanin (APC)–, APC-Cy7–, and Pacific blue–conjugated anti–Gr-1 (RB6-8C5), anti-CD117 (c-Kit; 2B8), anti-FcεRIα (MAR-1), anti-CD49b (Dx5), anti-CD69 (H1.2F3), anti-CD86 (GL1), anti-CD11b (M1/70), and anti-CD45.1 (A20) mAbs were purchased from eBioscience (San Diego, Calif). APC- and PE-conjugated anti–Siglec-F (E50-2440) mAbs were purchased from BD Biosciences (San Jose, Calif). Fluorescein isothiocyanate–conjugated anti–intercellular adhesion molecule 1 (CD54; 3E2) mAb was purchased from BD Biosciences (Franklin Lakes, NJ). Brilliant Violet-conjugated anti-CD45 (30-F11) and purified anti-CD200R3 (Ba13) mAbs and rat anti–mast cell serine protease 8 (TUG8) were purchased from BioLegend (San Diego, Calif). For fluorescence labeling, purified anti-CD200R3 mAb was labeled with the HiLyte Fluor 647 Labeling Kit (Dojindo, Kumamoto, Japan). Functional-grade purified anti-FcεRIα (MAR-1), anti–TNF-α (MP6-XT22), and anti–IL-4 (11B11) mAbs were purchased from eBioscience.

Single-cell suspensions from skin were prepared for flow cytometric analysis as follows. Skin/ear samples were collected by using 8-mm skin biopsy specimens that were cut into pieces and then digested for 1 hour at 37°C in 1.6 mg/mL collagenase type II (Worthington Biochemical, Freehold, NJ) and 0.1 mg/mL DNase I (Sigma-Aldrich) in complete RPMI medium (cRPMI; RPMI 1640 medium [Sigma-Aldrich] containing 10% heat-inactivated FCS [Invitrogen], 0.05 mmol/L 2-mercaptoethanol, 2 mmol/L L-glutamine, 25 mmol/L N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 1 mmol/L nonessential amino acids, 1 mmol/L sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin). Samples were passed through a 40-μm pore size nylon mesh, and cells were stained for the indicated markers. Samples were acquired on a FACSFortessa system (BD Biosciences) and analyzed with FlowJo software (TreeStar, San Carlos, Calif). The numbers of each cell subset were calculated by means of flow cytometry and presented as numbers per square millimeter of skin surface.

ICD and basophil-depletion models

Mice were anesthetized with diethyl ether, and 20 μL of 1% (vol/vol) croton oil in acetone was applied to ear skin. Mice were injected twice daily for 3 days with anti-FcεRIα (MAR-1) to deplete basophils *in vivo*.[22] The efficiency of basophil depletion was analyzed in peripheral blood on day 4. Mice were intraperitoneally injected with NAC (500 mg/kg body weight) and given 20 μL of 50 mmol/L NAC in 100% ethanol on ear skin 1 hour before application of croton oil to block ROS production.

Bas TRECK Tg mice were treated with DT for basophil depletion. BALB/c mice with DT were used as control animals.[20] For DT treatment, mice were injected intraperitoneally with 100 ng of DT per mouse.

Histology and immunohistochemistry

Skin samples for hematoxylin and eosin (H&E) staining were collected from patients with ICD (n = 10) and healthy control subjects (n = 6). The number of eosinophils was counted in 5 fields (20× objective). H&E staining and histologic scoring were evaluated, as previously reported.[23] In brief, samples were scored for the severity and character of the inflammatory response on a subjective grading scale. Responses were graded as follows: 0, no response; 1, minimal response; 2, mild response; 3, moderate response; and 4, marked response. The slides were blinded, randomized, and reread to determine the histologic score. All studies were read by the same pathologist by using the same subjective grading scale. The total histologic score was calculated as the sum of scores, including inflammation, neutrophils, mononuclear cells, edema, and epithelial hyperplasia. The evaluation of eosinophils was performed with Papanicolaou staining.

For the identification of basophils by means of immunohistochemistry, tissue sections were immunostained, as previously reported.[24]

Staining of ROS in ear skin

Mice were treated with 1% croton oil, and cells from the ear skin were isolated 6 hours later and incubated for 30 minutes at 37°C with a solution of 1 μmol/L CM-H_2DCFDA in PBS. After being washed twice with PBS, cells were labeled with anti–Siglec-F and anti-CD11b. We detected production of ROS, as indicated by an increase in 2′,7′-dichlorofluorescein fluorescence.