

Basophils preferentially express mouse mast cell protease 11 among the mast cell tryptase family in contrast to mast cells

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ABSTRACT

Tryptases and chymases are the major proteins stored and secreted by mast cells, and they have various biological functions. However, the nature of proteases produced by basophils has been poorly characterized, particularly in mice. mMCP-11 is the most recently discovered mast cell tryptase in mice and was originally identified as *Prss34*, which is transcribed in some mast cell-like cell lines and at the early stage in the culture of BMMC with IL-3. Curiously, *Prss34* is preferentially expressed in the BM and spleen among normal tissues in contrast to other mast cell tryptases. Therefore, it remains elusive what types of cells express mMCP-11 in vivo. Here, we show that mMCP-11 is highly expressed by primary basophils and to a much lesser extent, by some mast cells. *Prss34* transcripts were detected abundantly in primary and cultured basophils and very weakly in peritoneal mast cells or cultured BMMC. Conversely, transcripts for mMCP-6 and mMCP-7 tryptases were preferentially expressed by cultured and peritoneal mast cells but not basophils. We established a mMCP-11-specific mAb and showed that mMCP-11 proteins are indeed expressed by primary basophils and those infiltrating the affected tissues during allergic inflammation and parasitic infections. Some primary mast cells also expressed mMCP-11 proteins, albeit at a much lower level. Thus, basophils rather than mast cells are the major source of mMCP-11. This is the first study to demonstrate that mouse basophils produce a trypsin-like protease. *J. Leukoc. Biol.* **86**: 1417–1425; 2009.

Abbreviations: BMBA=bone marrow-derived basophil, BMMC=bone marrow-derived mast cell, *Cma1*=chymase 1, dMCP-3=dog MCP-3, *Hprt1*=hypoxanthine guanine phosphoribosyl transferase 1, IRES=internal ribosome entry site, *Isp-1/2*=implantation serine proteinase gene 1/2, mMCP-11=mouse mast cell protease 11, mTMT=mouse transmembrane tryptase, *Nb*=*Nippostrongylus brasiliensis*, *Prg2*=proteoglycan 2, *Prss34*=protease serine 34, SCF=stem cell factor, SD rat=Sprague Dawley rat, *Tpsa/b/g*=tryptase $\alpha/\beta/\gamma$, respectively

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

Introduction

Basophils are the least common granulocytes, representing <1% of peripheral blood leukocytes [1, 2]. Basophils circulate in the peripheral blood and do not migrate into the peripheral tissues under physiological conditions, whereas mature mast cells reside in the peripheral tissues and do not circulate in the peripheral blood. In spite of this difference in their anatomical localizations, circulating basophils share several features with the tissue-resident mast cells, including the presence of basophilic granules in the cytoplasm, the surface expression of the high-affinity IgE α , Fc ϵ RI, and the release of chemical mediators, such as histamine, upon stimulation. Owing to their similarities to mast cells and their small numbers, basophils have long been neglected in immunological studies or considered minor and possibly redundant “circulating mast cells”. Accordingly, basophils have often been studied as surrogates of the less-accessible tissue-resident mast cells in humans.

Recent studies using mouse models have revealed previously unrecognized roles for basophils in allergic responses and immune regulation and have shown that basophils and mast cells have distinct roles in immune responses [3–11]. Basophils play crucial roles in IgG-mediated systemic anaphylaxis [5] and IgE-mediated, chronic allergic inflammation [3, 4]. They also drive T cell differentiation from naïve to Th2-type cells [6, 9–11] and augment humoral memory responses [7]. These findings have changed our view of basophils markedly, from an uninteresting minority population to key players in immune regulation and allergic responses [12–14]. To understand further the molecular mechanisms by which basophils function in vivo under physiological and pathological conditions, it is important to identify effector molecules that are selectively expressed and secreted by basophils. Mast cells release an array of biologically active mediators, including neutral proteases and cytokines, in response to a variety of stimuli [1]. There is now firm evidence that basophils are a major source of Th2 cytokines [15–18]. However, the pro-

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duction and release of neutral proteases by basophils have been less well-studied, particularly in mice, compared with those by mast cells. Human basophils are reported to produce small amounts of α and β tryptases and little or no chymase [19–22]. However, it remains uncertain whether mouse basophils express any of the tryptases, particularly at the protein level.

Serine proteases with chymotrypsin-like (chymase) and trypsin-like (tryptase) properties are major constituents of mast cell granules [23, 24]. One chymase and four different tryptases (α , β , γ , δ) have been identified in human mast cells, and in mice, six mast cell chymases (mMCP-1, mMCP-2, mMCP-4, mMCP-5, mMCP-9, mMCP-10) and four mast cell tryptases (mMCP-6, mMCP-7, mMCP-11, mTMT) have been identified [23–25]. mMCP-11 is the most recently discovered member of the murine mast cell tryptase family [26]. The mMCP-11-encoding *Prss34* gene is located on mouse chromosome 17A3.3, which contains 13 genes encoding functional trypsin-like serine proteases, including the mast cell tryptases mMCP-6, mMCP-7, and mTMT. The *Prss34* gene was identified initially by probing the mouse expressed sequence tag database at GenBank with known tryptase cDNAs. The *Prss34* cDNA was subsequently isolated from BMMC cultured with IL-3 and found to encode a 318-aa polypeptide, including a signal peptide (19 aa), a propeptide (15 aa), and a mature form of protease (284 aa). *Prss34* mRNA was detected in two mast cell-like cell lines but in no fibroblast, T cell, or macrophage cell lines [26]. Of note, BMMC cultured with IL-3 show a transient expression of *Prss34* mRNA. That is, *Prss34* mRNA is abundant in BM cells cultured with IL-3 for 1 week, but its level decreases substantially after 3 weeks, suggesting that mMCP-11 might be expressed at the early stage of mast cell development [26]. Analysis of *Prss34* mRNA expression in various tissues revealed it to be preferentially expressed in the spleen and BM [26], which is different from the expression profile of other mast cell tryptases. Thus, it remains to be clarified what types of cells indeed express mMCP-11 in vivo.

In the present study, we first examined the expression of *Prss34* mRNA in primary hematopoietic cells and found that it was highly expressed in basophils and very weakly in mast cells. We then established a mMCP-11-specific mAb to examine the expression of mMCP-11 at the protein level. The mMCP-11 proteins were expressed abundantly by primary basophils and those infiltrating the affected tissues during allergic inflammation and parasitic infection, and they were expressed to a much lesser extent by some mast cells. Thus, basophils represent a more significant source of mMCP-11 than do mast cells.

MATERIALS AND METHODS

Animals

BALB/c and C57BL/6 mice (6–10 weeks old) and SD rats (8 weeks old) were purchased from CLEA Japan (Tokyo, Japan). C57BL/6-*Ki^{W-sh}/W-sh* mice [27] were maintained under a specific pathogen-free condition in our facility. All of the experiments were performed according to the Guidelines for Animal Use and Experimentation, as set by the Tokyo Medical and Dental University (Japan).

Cell lines, cell preparation, and culture

The cell lines X63.Ag8-653 (myeloma cell-derived, ATCC CRL 1580) and NIH3T3 (fibroblast-derived, ATCC CRL 8306) were cultured in IMDM (Gibco, Paisley, UK) and DMEM (Sigma-Aldrich, St Louis, MO, USA), respectively, supplemented with 10% FCS, 100 U/ml penicillin, 100 μ g/ml streptomycin, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM L-glutamine, and 5×10^{-5} M 2-ME. A retroviral packaging cell line Plat-E [28] was maintained in supplemented DMEM containing 1 μ g/ml puromycin (Sigma-Aldrich) and 10 μ g/ml blasticidin (Invitrogen Life Technology, Carlsbad, CA, USA). BMBA [6, 8–10, 29, 30] were prepared by culturing BM cells in the presence of rIL-3 [31] (300 pg/ml) for 1 week, followed by isolating the CD49b⁺ cells using the IMag system with biotinylated anti-CD49b and streptavidin-conjugated magnetic particles (BD PharMingen, San Diego, CA, USA). BMMC were prepared by culturing BM cells in the presence of rIL-3 (300 pg/ml), with or without rSCF (10 ng/ml, PeproTec, London, UK) for 5 weeks. Basophils (CD49b⁺Fc ϵ RI α ⁺), neutrophils (Gr-1^{high}SiglecF⁺), and eosinophils (Gr-1^{med}SiglecF⁺) in the BM and mast cells (Fc ϵ RI α ⁺ckit⁺) in the peritoneal cavity of BALB/c mice were sorted separately by using FACS Vantage (BD Biosciences, Mountain View, CA, USA). Purity of sorted cells was >95%.

Antibodies

Anti-FLAG (M2) mAb and M2 affinity gel were purchased from Sigma-Aldrich. Anti-mMCP-7 mAb and the HRP-conjugated goat anti-rat IgG and anti-mouse IgG antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Isotype-matched control antibodies (rat IgG2a κ and rat IgG2b κ), PE-conjugated anti-Siglec-F (E50-2440), biotinylated mAb specific to CD49b (DX5) and c-kit (2B8), and allophycocyanin-conjugated streptavidin were purchased from BD PharMingen. FITC-conjugated anti-CD49b (DX5), PE-conjugated anti-Fc ϵ RI α (MAR-1), and biotinylated anti-Gr-1 (RB6-8C5) were purchased from eBioscience (San Diego, CA, USA). HRP-conjugated rabbit anti-mMCP-1 antibody was from Moredun Scientific Ltd. (Penicuik, Scotland), and anti-mMCP-6 mAb was from R&D Systems (Minneapolis, MN, USA).

Establishment of mAb specific to mMCP-8 or mMCP-11

The coding region of the mMCP-8 or mMCP-11 cDNA was subcloned into the expression vector pET41a(+) (Novagen, Darmstadt, Germany), which was constructed to produce fusion proteins consisting of GST, His-tag, and the protein of interest when expressed in *Escherichia coli*. rGST-mMCP-8 and rGST-mMCP-11 fusion proteins were purified from *E. coli* lysates by using a Ni-column, HisTrapTM high performance (GE Healthcare Bio-sciences AB, Uppsala, Sweden). SD rats were immunized at weekly intervals by footpad injection of GST-mMCP-8 (100 μ g) or GST-mMCP-11 (500 μ g). CFA (Sigma-Aldrich) and IFA (Sigma-Aldrich) were used as adjuvants for the first immunization and subsequent four booster immunizations, respectively. Three days after the sixth immunization, which was without adjuvant, cells were isolated from the popliteal lymph nodes and fused with X63.Ag8-653 cells using polyethylene glycol 1500 (Roche Diagnostics, Indianapolis, IN, USA). B cell hybridomas were selected in medium containing hypoxanthine/aminopterin/thymidine (Gibco-BRL, Carlsbad, CA, USA) and rIL-6 [32]. Their culture supernatants were screened for the ability to react with mMCP-8 or mMCP-11 by ELISA, in which 96-well plates coated with GST, GST-mMCP-8, or GST-mMCP-11 were reacted with hybridoma culture supernatant followed by anti-rat IgG-HRP. Hybridoma clones showing the reactivity to GST-mMCP-8 or GST-mMCP-11 but not GST were selected.

Retroviral vectors and infection

The cDNAs of FLAG-tagged mMCP-8 and mMCP-11 were inserted into the retroviral vector pMX-IRES-GFP [28]. Plat-E cells were transfected with the vectors using FuGENE 6 (Roche Diagnostics), and their culture supernatants were collected 48 h later. NIH3T3 cells were infected by incubation with the Plat-E culture supernatant in the presence of 5 μ g/ml polybrene (Sigma-Aldrich) for 48 h.

Semiquantitative RT-PCR analysis

Total RNA was prepared from cells and then subjected to first-strand cDNA synthesis with RT using oligo-dT primers. Semiquantitative PCR was performed with fivefold serially diluted cDNA templates. Intron-spanning primers were used to avoid coamplification of genomic DNA, as listed in **Table 1**. The expression of a housekeeping gene, *Hprt1*, was analyzed in parallel, as a reference for normalization.

Immunoprecipitation and immunoblotting

Cells were lysed in lysis buffer containing 0.5% Triton X-100 (Sigma-Aldrich), protease inhibitor cocktail (Roche Diagnostics), and 300 μ M diisopropylfluorophosphate (Sigma-Aldrich). Cell lysates or culture supernatants were incubated with an anti-FLAG affinity gel, and the immunoprecipitates were subjected to SDS-PAGE (12% polyacrylamide), followed by immunoblotting with the indicated antibodies and HRP-conjugated secondary antibodies. Proteins were visualized by Immobilon Western (Millipore, Billerica, MA, USA).

Histological and flow cytometric analyses

For immunohistochemical examination, tissues were fixed with 10% buffered formalin, embedded in paraffin, and cut with a microtome into 3 μ m-thick sections. They were treated with microwaves, followed by incubation with methanol containing 0.3% H₂O₂ to inhibit endogenous peroxidase and with a protein-blocking solution containing 0.25% casein (Dako, Carpinteria, CA, USA) to prevent the nonspecific binding of antibodies. Cytospin preparations were fixed with methanol for 10 min. Tissue sections and cytospin preparations were incubated with the indicated antibodies (1 μ g/ml) at 4°C overnight, followed by HRP-conjugated goat anti-rat IgG (diluted 1:500). They were incubated subsequently in 3'-diaminobenzidine tetrahydrochloride solution (Dako) and counterstained with hematoxylin. For flow cytometric analysis, single-cell suspensions were incubated with the indicated antibodies after treatment with an anti-CD16/32 mAb (2.4G2)

and normal rat serum to reduce the nonspecific staining. Cells were analyzed with a FACSCalibur (BD Biosciences).

IgE-mediated, chronic allergic skin inflammation

IgE-mediated, chronic allergic inflammation in the skin was induced as described previously [3, 4]. Briefly, mice were sensitized with an i.v. injection of 300 μ g TNP-specific IgE (ATCC TIB 141) 1 day before being challenged with a s.c. injection of 10 μ g TNP-conjugated OVA (BioResearch Technologies, Novato, CA, USA) or control OVA in the ear lobes.

Nb infection

Mice were infected with Nb by s.c. inoculation of 500 third-stage larvae.

RESULTS

Prss34 transcripts are highly expressed in IL-3-differentiated basophils and barely in IL-3-differentiated mast cells

mMCP-11-encoding *Prss34* transcripts were detected readily by RT-PCR in murine BM cells that had been cultured with IL-3 for 1 week and to a much lesser extent, in those cultured for 5 weeks but rarely in those cultured with IL-3 plus SCF for 5 weeks (**Fig. 1A**, lower panel). These results agreed with the original report on *Prss34*, in which this expression pattern was interpreted as a preferential expression of *Prss34* at an early stage of mast cell development [26]. Importantly, basophils are also included in the short-term culture of BM cells in IL-3 [29] and rare in the long-term culture, in contrast to mast cells. Under our culture conditions, basophil-type cells (CD49b⁺c-kit⁺)

TABLE 1. Primer Sets Used for RT-PCR Analysis

Gene	Gene product	Direction	PCR primers	Product size (bp)
<i>Prss34</i>	mMCP-11	Forward	5'-GCTGATGAAAGTGCTCAAGATCATCCG-3'	610
		Reverse	5'-AGGAGTGAATGGATCAATATGAGTGGCTG-3'	
<i>Mcpt8</i>	mMCP-8	Forward	5'-CCGGAATTCATGTTCTGCTCCTGGTCC-3'	763
		Reverse	5'-CCGGATCCCCTAGGTTGTTGCAGGAGTTTCATTG-3'	
<i>Mcpt1</i>	mMCP-1	Forward	5'-ATGCAGGCCCTACTATTCCTGATGG-3'	741
		Reverse	5'-CTACTTGCCATTTATGACTGTTTTAATCC-3'	
<i>Mcpt2</i>	mMCP-2	Forward	5'-GGGCTGGAGCTGAGGAGATT-3'	714
		Reverse	5'-GACTCAGGCTGGTTAGGCTT-3'	
<i>Mcpt4</i>	mMCP-4	Forward	5'-GGGCTGGAGCTGAGGAGATT-3'	224
		Reverse	5'-CTTCTGCTGTGTGGATTCTGTC-3'	
<i>Cma1</i>	mMCP-5	Forward	5'-ATGAAGCCTGCAGCAGCCCTGAGG-3'	783
		Reverse	5'-TTAATTCTCCTCAAGATCTTATTGATCC-3'	
<i>Tpsb2</i>	mMCP-6	Forward	5'-CCGGAATCCAGCCAATCAGCGAGTGGGCAT-3'	776
		Reverse	5'-CCGCTCGAGTCAGGAATGCTCAGGGACATAGCGG-3'	
<i>Tpsab1</i>	mMCP-7	Forward	5'-ATGACCACCTGATGACTGTGAGCCAG-3'	563
		Reverse	5'-AGGAACGGAGGTCATCTGGATGTG-3'	
<i>Mcpt9/Cma2</i>	mMCP-9/10	Forward	5'-GGTTATGTGGCAATCTGTGG-3'	528
		Reverse	5'-GCGTCCAGAAGATAACAATACC-3'	
<i>Tpsg1</i>	mTMT	Forward	5'-GATCATCATGTACTGGCTCTCCAG-3'	645
		Reverse	5'-CTACACCTCATTGAGGTTCCGAGG-3'	
<i>Mmp9</i>	MMP-9	Forward	5'-GGTACTGGAAGTTCCTGAATCATAGA-3'	409
		Reverse	5'-CAGAAATAGGCTTTGCTCTTGTTACTG-3'	
<i>Prg2</i>	MBP	Forward	5'-GGAGCGTCTGCTCTTCATCT-3'	501
		Reverse	5'-ACTTCCATCAACCCATCGAA-3'	
<i>Hprt1</i>	HPRT	Forward	5'-GCTGGTGAAAAGGACCTCT-3'	249
		Reverse	5'-CACAGGACTAGAACACCTGC-3'	

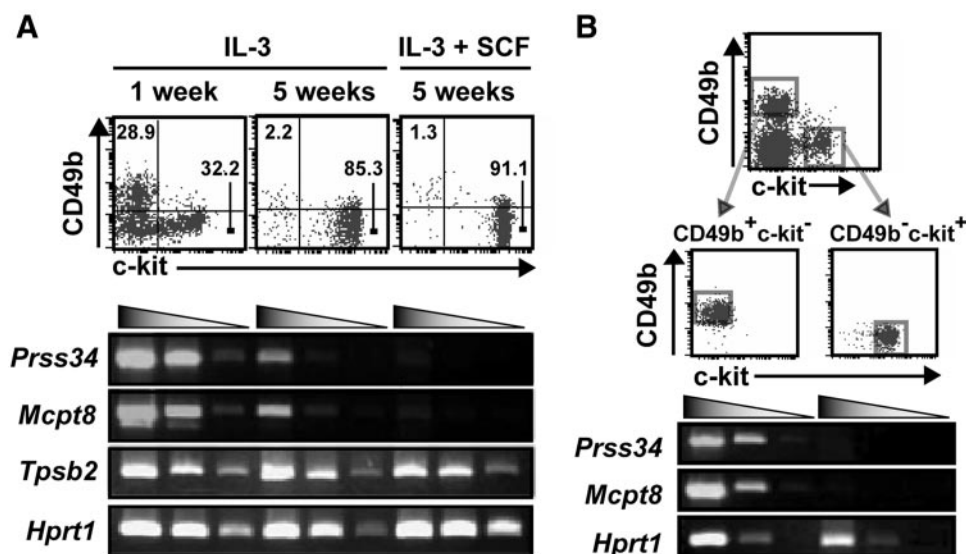


Figure 1. *Prss34* transcripts are detected in cultured basophils but barely in cultured mast cells. (A) BALB/c BM cells were cultured with IL-3 for 1 or 5 weeks or with IL-3 plus SCF for 5 weeks, and the cultured cells were stained for the surface expression of c-kit and CD49b (upper panel). RNAs, prepared from the cultured cells, were subjected to semiquantitative RT-PCR analysis for the expression of the indicated genes (lower panel). The PCR templates were fivefold serially diluted, as symbolized by gradation arrows above the lower panel. (B) CD49b⁺c-kit⁻ and CD49b⁻c-kit⁺ cells in BM cells cultured with IL-3 for 1 week were sorted separately and subjected to RT-PCR analysis as in A. Data shown in A and B are representative of three independent experiments using cells prepared from different mice.

[3–11, 17, 30, 33] accounted for ~30%, 2–5%, and <2% of the cultured BM cells in the 1- and 5-week cultures with IL-3 and the 5-week culture with IL-3 plus SCF, respectively, and mast cell-type cells (CD49b⁻c-kit⁺) represented ~30%, 80–85%, and >90% of the cultured cells, respectively (Fig. 1A, upper panel). Both types of cells expressed FcεRI on their surface (data not shown). Basophil-specific *Mcpt8* transcripts [34–36] were readily detected in the 1-week-cultured BM cells, in accordance with the presence of basophil-type cells. Their levels declined as the frequency of basophil-type cells was reduced during culture, and the level of the mast cell-specific *Tpsb2* transcripts encoding mMCP-6 remained unchanged (Fig. 1A, lower panel). Thus, the time-course of the *Prss34* transcription during culture was similar to that of the *Mcpt8* transcription, suggesting that *Prss34* might be expressed in basophils rather than mast cells, such as *Mcpt8*. To explore this possibility, CD49b⁺c-kit⁻ basophil-type cells and CD49b⁻c-kit⁺ mast cell-type cells were sorted separately from 1-week-cultured BM cells and subjected to RT-PCR analysis (Fig. 1B). The *Prss34* and *Mcpt8* transcripts were detected in the basophil-type cells but hardly in the mast cell-type cells.

Primary basophils express *Prss34* transcripts at a higher level than do peritoneal mast cells

We next examined the expression of *Prss34* transcripts in primary cells, including basophils, mast cells, neutrophils, and eosinophils. *Prss34* transcripts were readily detected in CD49b⁺FcεRI⁺ basophils [3–11, 17, 30, 33], that were freshly isolated from the BM, weakly in peritoneal mast cells and hardly at all in neutrophils and eosinophils, as were the *Mcpt8* transcripts (Fig. 2). No significant expression of *Prss34* mRNA was detected in the other hematopoietic cells examined, including T cells, B cells, NK cells, monocytes, macrophages, and dendritic cells (data not shown). In contrast to the *Prss34*

transcripts, the *Tpsb2* and *Tpsab1* transcripts, encoding the mast cell tryptases mMCP-6 and mMCP-7, respectively, were readily detected in the peritoneal mast cells but barely in ba-

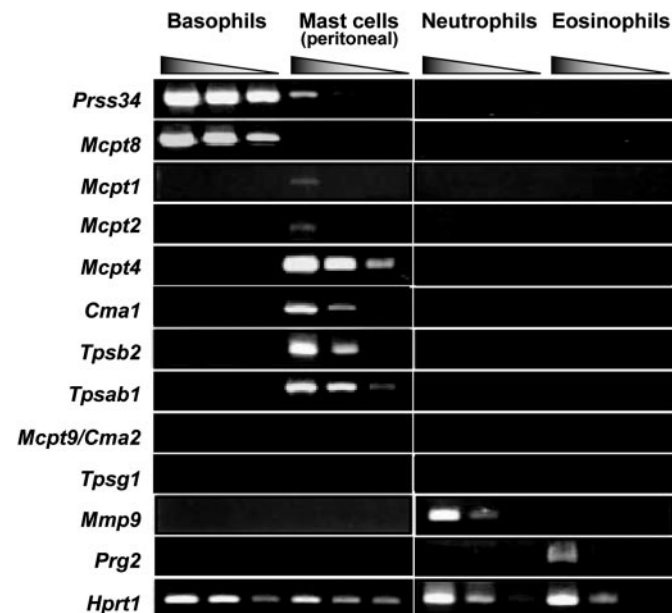


Figure 2. Primary basophils predominantly express *Prss34* transcripts among the mast cell tryptases, whereas peritoneal mast cells mainly express *Tpsb2* and *Tpsab1* transcripts. Basophils (CD49b⁺FcεRI⁺), neutrophils (Gr-1^{high}SiglecF⁻), and eosinophils (Gr-1^{med}SiglecF⁺) in the BM and mast cells (FcεRI⁺c-kit⁺) in the peritoneal cavity of BALB/c mice were sorted separately, and their RNAs were subjected to semiquantitative RT-PCR analysis for the expression of the indicated genes. Data are representative of three independent experiments using cells prepared from different mice.

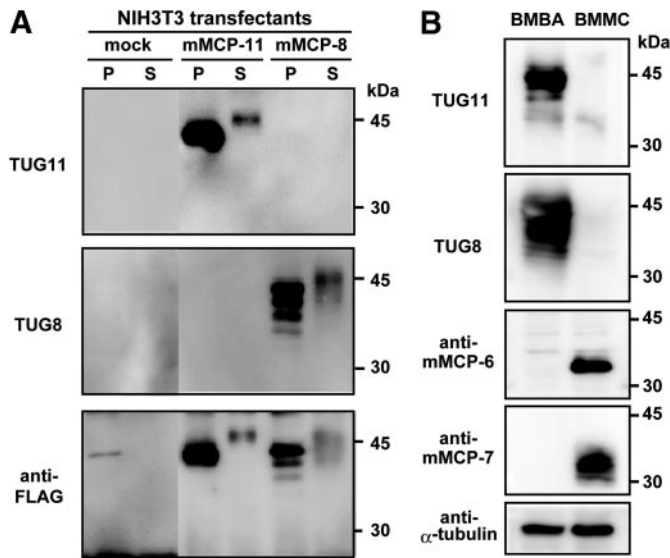


Figure 3. BMBA but not BMMC express mMCP-11 proteins detected by a newly established mMCP-11-specific mAb. (A) NIH3T3 cells were infected with a retroviral vector containing a cDNA-encoding, FLAG-tagged mMCP-11 or mMCP-8 or vector alone (mock). Forty-eight hours later, the FLAG-tagged proteins were isolated from the lysates of cell pellets (P) and from the culture supernatants (S) and subjected to SDS-PAGE, followed by immunoblotting with the indicated antibodies. (B) Cell lysates were prepared from BMBA (CD49b⁺ fraction of BM cells cultured with IL-3 for 1 week) and BMMC (BM cells cultured with IL-3 for 5 weeks) and subjected to SDS-PAGE, followed by immunoblotting with the indicated antibodies. Data shown in A and B are representative of five and three experiments, respectively.

sophils, neutrophils, and eosinophils (Fig. 2). Neither the basophils nor the peritoneal mast cells expressed any detectable level of *Tpsg1*, which encodes the mucosal mast cell tryptase mTMT [37] (Fig. 2). No mast cell chymase transcripts (*Mcpt1*, *Mcpt2*, *Mcpt4*, *Cma1/Mcpt5*, *Mcpt9*, *Cma2/Mcpt10*) were detected in basophils (Fig. 2).

mMCP-11 proteins are produced by cultured and primary basophils

To examine mMCP-11 expression in basophils at the protein level, we established a mMCP-11-specific mAb (IgG2b, κ), des-

ignated as TUG11, by immunizing rats with rmMCP-11. As a positive control for the identification of mouse basophils, we also established a mMCP-8-specific mAb (IgG2a, κ), TUG8. mMCP-8 is a basophil-specific, granzyme B-like protease consisting of 227 aa in its mature form and is distinct from the classical tryptases and chymases in mast cells [34–36]. Antiserum against mMCP-8 has been produced [35], and no mAb specific to mMCP-8 has been reported to our knowledge. TUG11 detected 42 and 45 kDa proteins in cell lysates and culture supernatants, respectively, of mMCP-11-expressing but not mMCP-8-expressing NIH3T3 transfectants (Fig. 3A), consistent with the previous study using human embryonic kidney 293T transfectants [26]. TUG8 detected 38–43 kDa and 40–45 kDa proteins in the cell lysates and culture supernatants, respectively, of mMCP-8-expressing but not mMCP-11-expressing NIH3T3 transfectants (Fig. 3A). Previous studies using N-glycosidase F showed that mMCP-8 and mMCP-11 proteins are modified with N-linked glycosylation [26, 38]. In particular, mMCP-8 proteins possess six potential N-linked glycosylation sites [34], and the N-glycosidase F treatment reduced their apparent molecular weight from 42–44 kDa to 30 kDa [38]. Thus, the difference in the apparent molecular weights of the mMCP-11 and mMCP-8 proteins detected in the cell lysates and supernatants of the NIH3T3 transfectants is likely a result of a difference in the extent of glycosylation.

We next used the established mAb to examine the expression of the mMCP-11 and mMCP-8 proteins in BMBA (the CD49b⁺ fraction of BM cells cultured with IL-3 for 1 week) [6, 8–10, 29, 30] and BMMC (BM cells cultured with IL-3 for 5 weeks). As expected, the immunoblot with TUG8 detected mMCP-8 proteins with an apparent molecular weight of 38–45 kDa in BMBA but hardly anything in BMMC (Fig. 3B). Conversely, anti-mMCP-6 and anti-mMCP-7 mAb detected 35 kDa and 30–35 kDa proteins, respectively, in BMMC but not in BMBA (Fig. 3B). Of note, TUG11 detected major protein bands of 42–45 kDa in BMBA but barely anything in BMMC (Fig. 3B). The expression of mMCP-11 proteins was demonstrated further by subjecting cytospin samples of BMBA to immunoenzymatic staining with TUG11 (Fig. 4A). mMCP-11 and mMCP-8 proteins were detected in the cytoplasm of BMBA in a granular pattern (Fig. 4A). Most of the BMMC were negative for mMCP-11 and mMCP-8 staining (Fig.

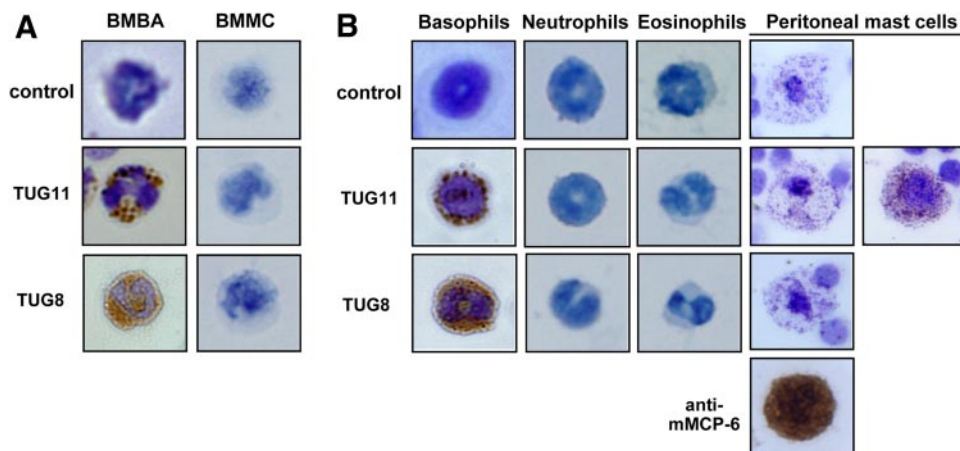


Figure 4. Primary basophils express mMCP-11 proteins. Cytospin preparations of BMBA and BMMC (A), basophils, neutrophils, and eosinophils isolated from the BM (B), and peritoneal mast cells (B) were stained with TUG11, TUG8, anti-mMCP-6, or isotype-matched control mAb (rat IgG2b κ and IgG2a κ) using the immunoenzymatic method. Staining with the IgG2b κ control mAb is shown in top panels, and comparable negative staining results were also obtained when the IgG2a κ control mAb was used (data not shown). Data are representative of three experiments.

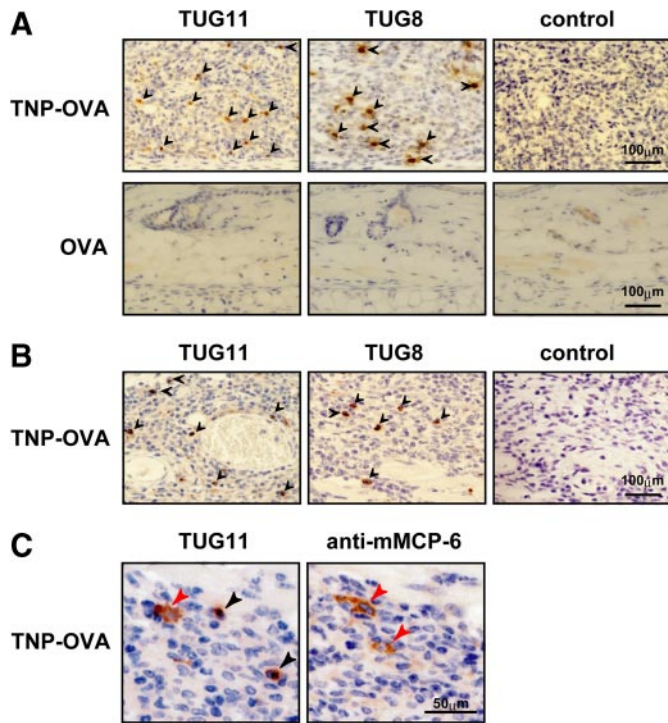


Figure 5. Basophils infiltrating the skin lesions of IgE-mediated, chronic, allergic inflammation express mMCP-11 proteins. Mast cell-deficient (C57BL/6-*Ki^{wsh/wsh}*) mice (A) and wild-type C57BL/6 mice (B and C) were passively sensitized with TNP-specific IgE and then challenged 1 day later by a s.c. injection of TNP-OVA or control OVA into the ear skin. Four days after the antigen challenge, the ear skin was subjected to the immunohistochemical examination using TUG11, TUG8, anti-mMCP-6, or isotype-matched control mAb (rat IgG2b κ and IgG2a κ). Arrowheads indicate stained cells (black for basophils and red for mast cells). Staining with the IgG2b κ control mAb is shown, and comparable negative staining results were also obtained when the IgG2a κ control mAb was used (data not shown). Data shown are representative of five independent experiments.

4A), although ~10% of them showed TUG11 or TUG8 staining to a variable extent (data not shown).

Virtually all basophils that were freshly isolated from the BM of BALB/c mice (Fig. 4B) and C57BL/6 mice (data not presented) showed staining, not only by TUG8 but also by TUG11, clearly demonstrating that the mMCP-11 proteins were expressed in primary basophils. In accordance with their granular staining pattern (Fig. 4B), mMCP-11 and mMCP-8 proteins were largely localized to the secretory granules of primary basophils, as detected by immunoelectron microscopy (data not shown). Neutrophils and eosinophils were negative for mMCP-11 as for mMCP-8 (Fig. 4B). Peritoneal mast cells were heavily stained with anti-mMCP-6, whereas none of them were detectably stained with TUG8 (Fig. 4B). Approximately 70% of peritoneal mast cells were negative for the TUG11 staining under the same conditions as for basophil staining (Fig. 4B, left panel of TUG11 staining), and the rest of them were faintly stained with TUG11 (Fig. 4B, right panel of TUG11 staining). These results demonstrated that mMCP-11 proteins are highly expressed by virtually all basophils, as are

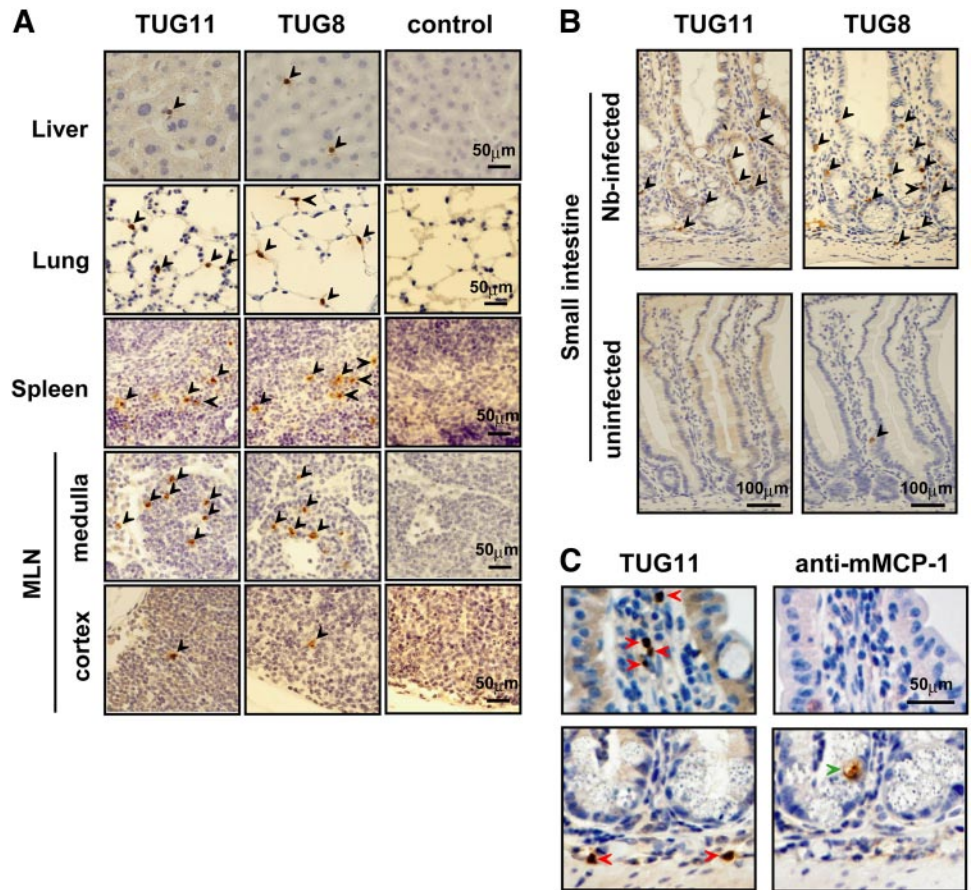
mMCP-8 proteins, but that mMCP-11 is not as specific to basophils as mMCP-8, and some peritoneal mast cells produce mMCP-11, albeit at a much lower level than do basophils.

Expression of mMCP-11 proteins in basophils infiltrating affected tissues during allergic inflammation and parasitic infection

We next examined the expression of mMCP-11 proteins in tissue-infiltrating basophils by means of the immunohistochemistry with TUG11. We reported previously that basophils play a crucial role in the initiation of IgE-mediated, chronic, cutaneous allergic inflammation, even though basophils account for only 2% of the infiltrating cells in skin lesions, as judged by flow cytometric analysis [3, 4]. This allergic inflammation can be elicited even in mast cell-deficient mice such as C57BL/6-*Ki^{wsh/wsh}* mice [3]. Immunohistochemical examination of ear skin specimens from C57BL/6-*Ki^{wsh/wsh}* mice demonstrated that TUG8⁺ basophils indeed infiltrated the dermis of skin challenged with relevant antigen TNP-OVA but not control OVA (Fig. 5A), in accordance with the result obtained from flow cytometric analysis [3, 4]. TUG11⁺ cells were also detected in the dermis in a pattern similar to TUG8⁺ basophils (Fig. 5A), suggesting that mMCP-11 proteins were expressed by skin-infiltrating basophils. In the skin lesions of mast cell-sufficient C57BL/6 mice, TUG11⁺ and TUG8⁺ cells were also detected, and none of them was stained with isotype-matched control antibodies (Fig. 5B). Of note, two types of TUG11⁺ cells, relatively small and large ones (indicated by black and red arrowheads, respectively, in Fig. 5C), were detected in the skin lesions of C57BL/6 mice, whereas virtually all of the TUG8⁺ cells were relatively small. Examination of serial sections revealed that the larger TUG11⁺ cells were positive for mMCP-6, and the smaller ones were negative for mMCP-6 (Fig. 5C). Therefore, the larger and smaller cells were likely mast cells and basophils, respectively. The frequency of TUG11⁺ staining among mMCP-6⁺ mast cells was on average 10–30% but varied, largely depending on the length of immunohistochemical reaction with substrates. In general, mast cells needed a much longer time to become brightly stained with TUG11 compared with basophils, suggesting the lower level of mMCP-11 expression in tissue-resident mast cells than in infiltrating basophils during the allergic inflammation.

Previous studies using flow cytometry demonstrated that the infection of mice with the nematode Nb induces basophil recruitment into the liver, lung, and intestine and increased numbers of basophils in the BM and spleen [8, 17, 18]. We therefore performed an immunohistochemical examination to know whether mMCP-11 proteins were expressed by tissue-infiltrating basophils in Nb-infected BALB/c mice. TUG11⁺ cells were detected in the sinusoids of the liver, alveolar walls in the lung, and red pulps of the spleen, just as were TUG8⁺ basophils (Fig. 6A). Isotype-matched control mAb did not react with any of the cells, and cells positive for TUG11 and TUG8 were rarely detected in the corresponding tissues from uninfected mice, except the spleen (data not shown), in accordance with our flow cytometric analysis. Comparable results were obtained when mast cell-deficient mice, with or without Nb infection, were analyzed (Supplementary Fig. 1; not all data shown). These results strongly suggested that basophils

Figure 6. Basophils infiltrating various tissues of Nb-infected mice express mMCP-11 proteins. BALB/c mice were infected with Nb (A, C, and B, upper panels) or left uninfected (B, lower panels). Ten days later, the liver, lung, spleen, mesenteric lymph nodes (MLN; A), and small intestine (B and C) were isolated and subjected to immunohistochemical examination using TUG11, TUG8, anti-mMCP-1, or isotype-matched controls. Arrowheads indicate stained cells (black for basophils in A and B; and red for basophils and green for mast cells in C). Data shown are representative of three independent experiments.



infiltrating the affected tissues during Nb infection expressed mMCP-11. Intriguingly, TUG11⁺ and TUG8⁺ cells were detected in the mesenteric lymph nodes of Nb-infected but not uninfected mice, predominantly in the medulla, although a few positive cells were in the cortex (Fig. 6A). In the small intestine, TUG11⁺ and TUG8⁺ cells were detected frequently in the lamina propria of Nb-infected mice but rarely in that of uninfected mice (Fig. 6B). This implied that mucosal mast cells express no detectable level of mMCP-11 and mMCP-8, as they reside in the lamina propria, even in the uninfected mice. In serial sections, mMCP-1⁺ mast cells were detected, but the localization of mMCP-1⁺ and TUG11⁺ cells was seldom overlapped (Fig. 6C). Moreover, RT-PCR analysis failed to detect the *Prss34* expression in the intestine from uninfected mice, although mTMT-encoding transcripts [37] were readily detected (data not shown). These results strongly suggested that basophils infiltrating the small intestine expressed mMCP-11, whereas the vast majority of mucosal mast cells was negative for mMCP-11, even during Nb infection.

DISCUSSION

The present study demonstrated that primary basophils produce mMCP-11 proteins. This is the first study, to our knowledge, to show that mouse basophils produce a trypsin-like protease. Some primary mast cells also produce mMCP-11 pro-

teins, but the level of the expression is much lower than in basophils under the conditions tested. Thus, basophils represent a more significant source of mMCP-11 than do mast cells. The *Prss34* gene was isolated originally from IL-3-cultured BM cells and therefore, reported in association with mast cells and their progenitors [26]. Of note, the RT-PCR analysis demonstrated that the *Prss34* transcripts are preferentially expressed in the BM and spleen among the organs and tissues tested, unlike the expression profile of other mast cell tryptases [26]. This observation could well be explained by the preferential expression of *Prss34* in primary basophils, as the basophil content of these two organs is higher than that of others.

The mouse mast cell tryptases can be categorized into two groups: membrane-anchored (mTMT) and soluble (mMCP-6, mMCP-7, mMCP-11) [23]. Among the three soluble tryptases, mMCP-11 is the most distantly related to the others [26]. The amino acid sequence of mMCP-11 is 61% identical to that of dog mastin/dMCP-3, which is expressed by mast cells and neutrophils in dogs [39–41]. Mouse and rat mMCP-11, dog mastin/dMCP-3, and pig TC30 form an orthologous clade that is distinguished readily from other soluble tryptases, including classical mast cell tryptases and implantation serine proteases *Prss28*/Isp-1 and *Prss29*/Isp-2 [26, 42]. Consistent with this analysis, using a panel of chromogenic substrates revealed that mMCP-11 displays trypsin-like activity with a substrate specificity distinct from that of other tryptases [26]. mMCP-6 and

mMCP-7 are selectively expressed in connective tissue-type mast cells [43, 44], whereas the *Tpsg1* transcripts, which encode mTMT, are detected predominantly in the intestine [37], most likely in mucosal-type mast cells. We demonstrated in this study that mMCP-11 is highly expressed by basophils and to a lesser extent, by some mast cells in the peritoneal cavity and skin but rarely by mucosal mast cells in the intestine. The distinct preference of individual members of the mast cell tryptase family in the cellular and tissue expression strongly suggests that they function differentially in vivo.

Among the mouse mast cell tryptases, mMCP-6 and mMCP-7 are characterized most extensively in terms of their functions [45, 46]. mMCP-7 exhibits anticoagulant activity through the degradation of fibrinogen [44]. The i.p. administration of mMCP-7 to mice results in the selective extravasation of eosinophils [47], whereas mMCP-6 administration results in the selective extravasation of neutrophils [48]. mMCP-6 induces endothelial cells to express large amounts of the neutrophil-attracting chemokine CXCL8/IL-8 [48]. The analysis of mMCP-6-deficient mice revealed that mMCP-6 has an immunoprotective role in bacterial and parasitic infections [49, 50] and is also involved in the pathogenesis of experimental arthritis in the K/BxN mouse model [51]. Although the physiological substrates of mMCP-11 remain to be determined, as for that of mMCP-6, the basophil's preferential expression of mMCP-11 documented in the present study would provide an important clue to understand physiological and pathological functions of mMCP-11. We demonstrated previously that basophils play a crucial role in the development of IgE-mediated, chronic, cutaneous allergic inflammation as initiators rather than effectors [3, 4]. The in vivo depletion of basophils during the progression of the dermatitis results in decreased numbers of eosinophils and neutrophils infiltrating the skin lesions, concomitantly with the elimination of basophils from the lesions, suggesting that basophils are involved in the recruitment of eosinophils and neutrophils [4]. It is intriguing to speculate that the tryptase mMCP-11 and/or the granzyme B-like protease mMCP-8 released from activated basophils are involved in the recruitment of proinflammatory cells, such as eosinophils and neutrophils, during IgE-mediated, chronic, allergic inflammation. Future studies, including the establishment of mice deficient for mMCP-11 and/or mMCP-8, will be needed to verify this scenario.

The detection of basophils has been difficult, particularly in mice [52], as mouse basophils have fewer basophilic granules than human basophils, and this has sometimes led to the erroneous conclusion that basophils may not exist in mice [53]. Histochemical staining with basic dyes is generally unsuitable for detecting mouse basophils in formalin-fixed, paraffin-embedded tissue sections [54–56]. We demonstrated in the present study that the newly established anti-mMCP-11 and anti-mMCP-8 mAb successfully detected basophils in methanol-fixed cytospin preparations and in formalin-fixed, paraffin-embedded tissue sections. Therefore, these mAb would be useful for the immunohistochemical identification of tissue-infiltrating basophils. Notably, by using the mAb, we detected basophils in the mesenteric lymph nodes of Nb-infected but not uninfected mice. Recent studies demonstrated that basophils were transiently recruited into the draining lymph nodes after the s.c. administration of protease allergens, such as papain [6, 9] or eggs of *Schistosoma mansoni*

[11], leading to the differentiation of Th2 cells. Therefore, the detection of basophils in mesenteric lymph nodes of Nb-infected mice may suggest that basophils are involved in Th2 cell differentiation during the infection with Nb.

In conclusion, we demonstrated in the present study that the tryptase mMCP-11 is highly expressed by primary basophils and to a lesser extent, by some mast cells, unlike other mast cell tryptases. This would provide novel insight into the in vivo roles for mMCP-11 and basophils, including roles in allergic inflammation, protection against pathogens, and tissue remodeling.

AUTHORSHIP

T. U., T. K., K. M., and K. O. performed experiments, analyzed data, and contributed to the writing of the manuscript. Y. K., Y. M., Y. E., and H. Y. discussed the experimental strategy and participated in the data analysis and the writing of the manuscript. H. K. defined the experimental strategy, supervised and interpreted the experiments, and contributed to the writing of the manuscript.

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DISCLOSURE

The authors have no conflicting financial interests.

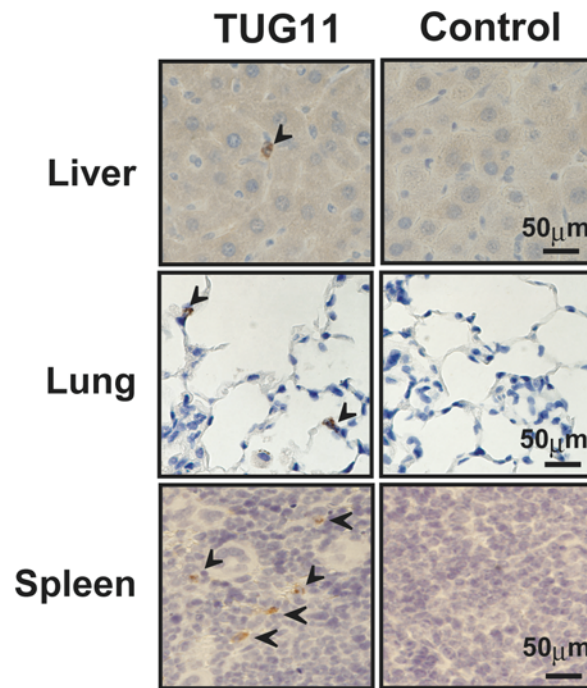
REFERENCES

- Galli, S. J. (2000) Mast cells and basophils. *Curr. Opin. Hematol.* **7**, 32–39.
- Falcone, F. H., Haas, H., Gibbs, B. F. (2000) The human basophil: a new appreciation of its role in immune responses. *Blood* **96**, 4028–4038.
- Mukai, K., Matsuoka, K., Taya, C., Suzuki, H., Yokozeki, H., Nishioka, K., Hirokawa, K., Etori, M., Yamashita, M., Kubota, T., Minegishi, Y., Yonekawa, H., Karasuyama, H. (2005) Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. *Immunity* **23**, 191–202.
- Obata, K., Mukai, K., Tsujimura, Y., Ishiwata, K., Kawano, Y., Minegishi, Y., Watanabe, N., Karasuyama, H. (2007) Basophils are essential initiators of a novel type of chronic allergic inflammation. *Blood* **110**, 913–920.
- Tsujimura, Y., Obata, K., Mukai, K., Shindou, H., Yoshida, M., Nishikado, H., Kawano, Y., Minegishi, Y., Shimizu, T., Karasuyama, H. (2008) Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity* **28**, 581–589.
- Sokol, C. L., Barton, G. M., Farr, A. G., Medzhitov, R. (2008) A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* **9**, 310–318.
- Denzel, A., Maus, U. A., Rodriguez Gomez, M., Moll, C., Niedermeier, M., Winter, C., Maus, R., Hollingshead, S., Briles, D. E., Kunz-Schughart, L. A., Talke, Y., Mack, M. (2008) Basophils enhance immunological memory responses. *Nat. Immunol.* **9**, 733–742.
- Ohnmacht, C., Voehringer, D. (2009) Basophil effector function and homeostasis during helminth infection. *Blood* **113**, 2816–2825.
- Sokol, C. L., Chu, N. Q., Yu, S., Nish, S. A., Laufer, T. M., Medzhitov, R. (2009) Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat. Immunol.* **10**, 713–720.
- Yoshimoto, T., Yasuda, K., Tanaka, H., Nakahira, M., Imai, Y., Fujimori, Y., Nakanishi, K. (2009) Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4(+) T cells. *Nat. Immunol.* **10**, 706–712.

11. Perrigoue, J. G., Saenz, S. A., Siracusa, M. C., Allenspach, E. J., Taylor, B. C., Giacomini, P. R., Nair, M. G., Du, Y., Zaph, C., van Rooijen, N., Comeau, M. R., Pearce, E. J., Laufer, T. M., Artis, D. (2009) MHC class II-dependent basophil-CD4(+) T cell interactions promote T(H)2 cytokine-dependent immunity. *Nat. Immunol.* **10**, 697–705.
12. Min, B. (2008) Basophils: what they “can do” versus what they “actually do”. *Nat. Immunol.* **9**, 1333–1339.
13. Karasuyama, H., Mukai, K., Tsujimura, Y., Obata, K. (2009) Newly discovered roles for basophils: a neglected minority gains new respect. *Nat. Rev. Immunol.* **9**, 9–13.
14. Sullivan, B. M., Locksley, R. M. (2009) Basophils: a nonredundant contributor to host immunity. *Immunity* **30**, 12–20.
15. Seder, R. A., Paul, W. E., Dvorak, A. M., Sharkis, S. J., Kagey-Sobotka, A., Niv, Y., Finkelman, F. D., Barbieri, S. A., Galli, S. J., Plaut, M. (1991) Mouse splenic and bone marrow cell populations that express high-affinity Fc ϵ receptors and produce interleukin 4 are highly enriched in basophils. *Proc. Natl. Acad. Sci. USA* **88**, 2835–2839.
16. Schroeder, J. T., MacGlashan Jr., D. W., Lichtenstein, L. M. (2001) Human basophils: mediator release and cytokine production. *Adv. Immunol.* **77**, 93–122.
17. Voehringer, D., Shinkai, K., Locksley, R. M. (2004) Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* **20**, 267–277.
18. Min, B., Prout, M., Hu-Li, J., Zhu, J., Jankovic, D., Morgan, E. S., Urban Jr., J. F., Dvorak, A. M., Finkelman, F. D., LeGros, G., Paul, W. E. (2004) Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J. Exp. Med.* **200**, 507–517.
19. Xia, H. Z., Kepley, C. L., Sakai, K., Chelliah, J., Irani, A. M., Schwartz, L. B. (1995) Quantitation of tryptase, chymase, Fc ϵ RI α , and Fc ϵ RI γ mRNAs in human mast cells and basophils by competitive reverse transcription-polymerase chain reaction. *J. Immunol.* **154**, 5472–5480.
20. Castells, M. C., Irani, A. M., Schwartz, L. B. (1987) Evaluation of human peripheral blood leukocytes for mast cell tryptase. *J. Immunol.* **138**, 2184–2189.
21. Foster, B., Schwartz, L. B., Devouassoux, G., Metcalfe, D. D., Prussin, C. (2002) Characterization of mast-cell tryptase-expressing peripheral blood cells as basophils. *J. Allergy Clin. Immunol.* **109**, 287–293.
22. Jogie-Brahim, S., Min, H. K., Fukuoka, Y., Xia, H. Z., Schwartz, L. B. (2004) Expression of α -tryptase and β -tryptase by human basophils. *J. Allergy Clin. Immunol.* **113**, 1086–1092.
23. Caughey, G. H. (2007) Mast cell tryptases and chymases in inflammation and host defense. *Immunol. Rev.* **217**, 141–154.
24. Miller, H. R., Pemberton, A. D. (2002) Tissue-specific expression of mast cell granule serine proteinases and their role in inflammation in the lung and gut. *Immunology* **105**, 375–390.
25. Hunt, J. E., Friend, D. S., Gurish, M. F., Feyfant, E., Sali, A., Huang, C., Ghildyal, N., Stechschulte, S., Austen, K. F., Stevens, R. L. (1997) Mouse mast cell protease 9, a novel member of the chromosome 14 family of serine proteases that is selectively expressed in uterine mast cells. *J. Biol. Chem.* **272**, 29158–29166.
26. Wong, G. W., Yasuda, S., Morokawa, N., Li, L., Stevens, R. L. (2004) Mouse chromosome 17A3.3 contains 13 genes that encode functional tryptic-like serine proteases with distinct tissue and cell expression patterns. *J. Biol. Chem.* **279**, 2438–2452.
27. Grimbaldston, M. A., Chen, C. C., Piliponsky, A. M., Tsai, M., Tam, S. Y., Galli, S. J. (2005) Mast cell-deficient W-shash c-kit mutant Kit W-sh/W-sh mice as a model for investigating mast cell biology in vivo. *Am. J. Pathol.* **167**, 835–848.
28. Nosaka, T., Kawashima, T., Misawa, K., Ikuta, K., Mui, A. L., Kitamura, T. (1999) STAT5 as a molecular regulator of proliferation, differentiation and apoptosis in hematopoietic cells. *EMBO J.* **18**, 4754–4765.
29. Dvorak, A. M., Seder, R. A., Paul, W. E., Morgan, E. S., Galli, S. J. (1994) Effects of interleukin-3 with or without the c-kit ligand, stem cell factor, on the survival and cytoplasmic granule formation of mouse basophils and mast cells in vitro. *Am. J. Pathol.* **144**, 160–170.
30. Yoshimoto, T., Tsutsui, H., Tominaga, K., Hoshino, K., Okamura, H., Akira, S., Paul, W. E., Nakanishi, K. (1999) IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. *Proc. Natl. Acad. Sci. USA* **96**, 13962–13966.
31. Karasuyama, H., Melchers, F. (1988) Establishment of mouse cell lines which constitutively secrete large quantities of interleukin 2, 3, 4 or 5, using modified cDNA expression vectors. *Eur. J. Immunol.* **18**, 97–104.
32. Tohyama, N., Karasuyama, H., Tada, T. (1990) Growth autonomy and tumorigenicity of interleukin 6-dependent B cells transfected with interleukin 6 cDNA. *J. Exp. Med.* **171**, 389–400.
33. Kojima, T., Obata, K., Mukai, K., Sato, S., Takai, T., Minegishi, Y., Karasuyama, H. (2007) Mast cells and basophils are selectively activated in vitro and in vivo through CD200R3 in an IgE-independent manner. *J. Immunol.* **179**, 7093–7100.
34. Lutzel-Schwab, C., Huang, M. R., Kullberg, M. C., Aveskogh, M., Hellman, L. (1998) Characterization of mouse mast cell protease-8, the first member of a novel subfamily of mouse mast cell serine proteases, distinct from both the classical chymases and tryptases. *Eur. J. Immunol.* **28**, 1022–1033.
35. Poorafshar, M., Helmby, H., Troye-Blomberg, M., Hellman, L. (2000) mMCP-8, the first lineage-specific differentiation marker for mouse basophils. Elevated numbers of potent IL-4-producing and mMCP-8-positive cells in spleens of malaria-infected mice. *Eur. J. Immunol.* **30**, 2660–2668.
36. Lunderius, C., Hellman, L. (2001) Characterization of the gene encoding mouse mast cell protease 8 (mMCP-8), and a comparative analysis of hematopoietic serine protease genes. *Immunogenetics* **53**, 225–232.
37. Wong, G. W., Tang, Y., Feyfant, E., Sali, A., Li, L., Li, Y., Huang, C., Friend, D. S., Krilis, S. A., Stevens, R. L. (1999) Identification of a new member of the tryptase family of mouse and human mast cell proteases which possesses a novel COOH-terminal hydrophobic extension. *J. Biol. Chem.* **274**, 30784–30793.
38. Gallwitz, M., Enoksson, M., Hellman, L. (2007) Expression profile of novel members of the rat mast cell protease (rMCP)-2 and (rMCP)-8 families, and functional analyses of mouse mast cell protease (mMCP)-8. *Immunogenetics* **59**, 391–405.
39. Vanderslice, P., Craik, C. S., Nadel, J. A., Caughey, G. H. (1989) Molecular cloning of dog mast cell tryptase and a related protease: structural evidence of a unique mode of serine protease activation. *Biochemistry* **28**, 4148–4155.
40. Yezzi, M. J., Hsieh, I. E., Caughey, G. H. (1994) Mast cell and neutrophil expression of dog mast cell protease-3. A novel tryptase-related serine protease. *J. Immunol.* **152**, 3064–3072.
41. Raymond, W. W., Tam, E. K., Blount, J. L., Caughey, G. H. (1995) Purification and characterization of dog mast cell protease-3, an oligomeric relative of tryptases. *J. Biol. Chem.* **270**, 13164–13170.
42. Raymond, W. W., Sommerhoff, C. P., Caughey, G. H. (2005) Mastin is a gelatinolytic mast cell peptidase resembling a mini-proteasome. *Arch. Biochem. Biophys.* **435**, 311–322.
43. Reynolds, D. S., Stevens, R. L., Lane, W. S., Carr, M. H., Austen, K. F., Serafini, W. E. (1990) Different mouse mast cell populations express various combinations of at least six distinct mast cell serine proteases. *Proc. Natl. Acad. Sci. USA* **87**, 3230–3234.
44. Huang, C., Wong, G. W., Ghildyal, N., Gurish, M. F., Sali, A., Matsumoto, R., Qiu, W. T., Stevens, R. L. (1997) The tryptase, mouse mast cell protease 7, exhibits anticoagulant activity in vivo and in vitro due to its ability to degrade fibrinogen in the presence of the diverse array of protease inhibitors in plasma. *J. Biol. Chem.* **272**, 31885–31893.
45. Hallgren, J., Pejler, G. (2006) Biology of mast cell tryptase. An inflammatory mediator. *FEBS J.* **273**, 1871–1895.
46. McNeil, H. P., Adachi, R., Stevens, R. L. (2007) Mast cell-restricted tryptases: structure and function in inflammation and pathogen defense. *J. Biol. Chem.* **282**, 20785–20789.
47. Huang, C., De Sanctis, G. T., O'Brien, P. J., Mizgerd, J. P., Friend, D. S., Drazen, J. M., Brass, L. F., Stevens, R. L. (2001) Evaluation of the substrate specificity of human mast cell tryptase β I and demonstration of its importance in bacterial infections of the lung. *J. Biol. Chem.* **276**, 26276–26284.
48. Huang, C., Friend, D. S., Qiu, W. T., Wong, G. W., Morales, G., Hunt, J., Stevens, R. L. (1998) Induction of a selective and persistent extravasation of neutrophils into the peritoneal cavity by tryptase mouse mast cell protease 6. *J. Immunol.* **160**, 1910–1919.
49. Thakurda, S. M., Melicoff, E., Sansores-Garcia, L., Moreira, D. C., Petrova, Y., Stevens, R. L., Adachi, R. (2007) The mast cell-restricted tryptase mMCP-6 has a critical immunoprotective role in bacterial infections. *J. Biol. Chem.* **282**, 20809–20815.
50. Shin, K., Watts, G. F., Oettgen, H. C., Friend, D. S., Pemberton, A. D., Gurish, M. F., Lee, D. M. (2008) Mouse mast cell tryptase mMCP-6 is a critical link between adaptive and innate immunity in the chronic phase of *Trichinella spiralis* infection. *J. Immunol.* **180**, 4885–4891.
51. Shin, K., Nigrovic, P. A., Crish, J., Boilard, E., McNeil, H. P., Larabee, K. S., Adachi, R., Gurish, M. F., Gobeze, R., Stevens, R. L., Lee, D. M. (2009) Mast cells contribute to autoimmune inflammatory arthritis via their tryptase/heparin complexes. *J. Immunol.* **182**, 647–656.
52. Dvorak, A. M. (2000) The mouse basophil, a rare and rarely recognized granulocyte. *Blood* **96**, 1616–1617.
53. Urbina, C., Ortiz, C., Hurtado, I. (1981) A new look at basophils in mice. *Int. Arch. Allergy Appl. Immunol.* **66**, 158–160.
54. Irani, A. M., Huang, C., Xia, H. Z., Kepley, C., Nafie, A., Fouda, E. D., Craig, S., Zweiman, B., Schwartz, L. B. (1998) Immunohistochemical detection of human basophils in late-phase skin reactions. *J. Allergy Clin. Immunol.* **101**, 354–362.
55. Macfarlane, A. J., Kon, O. M., Smith, S. J., Zeibecoglou, K., Khan, L. N., Barata, L. T., McEuen, A. R., Buckley, M. G., Walls, A. F., Meng, Q., Humbert, M., Barnes, N. C., Robinson, D. S., Ying, S., Kay, A. B. (2000) Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. *J. Allergy Clin. Immunol.* **105**, 99–107.
56. Plager, D. A., Weiss, E. A., Kephart, G. M., Mochalar, R. M., Matsumoto, R., Checkel, J. L., Schwartz, L. B., Gleich, G. J., Leiferman, K. M. (2006) Identification of basophils by a mAb directed against pro-major basic protein 1. *J. Allergy Clin. Immunol.* **117**, 626–634.

KEY WORDS:

allergic inflammation · parasite infection · mMCP-6 · mMCP-7



Supplemental Figure 1. mMCP-11-expressing basophils infiltrating tissues in Nb-infected mast cell-deficient mice. Mast cell-deficient (C57BL/6-*Kit*^{w-sh/w-sh}) mice were infected with *N. brasiliensis*. Ten days later, the liver, lung, and spleen were isolated and subjected to immunohistochemical examination using TUG11 or isotype-matched control. Arrowheads indicate stained cells. Data shown are representative of three independent experiments.