Biochemical and Biophysical Research Communications 460 (2015) 261–266

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cysteine protease antigens cleave CD123, the α subunit of murine IL-3 receptor, on basophils and suppress IL-3-mediated basophil expansion

Hideto Nishikado [a], Tsutomu Fujimura [b], Hikari Taka [b], Reiko Mineki [b], Hideoki Ogawa [a], Ko Okumura [a], Toshiro Takai [a, *]

[a] Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan
[b] Laboratory of Proteomics and Biomolecular Science, BioMedical Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 17 February 2015
Available online 13 March 2015

Keywords:
Cysteine protease
Papain
Basophil
IL-3
CD123

ABSTRACT

Th2 type immune responses are essential for protective immunity against parasites and play crucial roles in allergic disorders. Helminth parasites secrete a variety of proteases for their infectious cycles including for host entry, tissue migration, and suppression of host immune effector cell function. Furthermore, a number of pathogen-derived antigens, as well as allergens such as papain, belong to the family of cysteine proteases. Although the link between protease activity and Th2 type immunity is well documented, the mechanisms by which proteases regulate host immune responses are largely unknown. Here, we demonstrate that the cysteine proteases papain and bromelain selectively cleave the α subunit of the IL-3 receptor (IL-3Rα/CD123) on the surface of murine basophils. The decrease in CD123 expression on the cell surface, and the degradation of the extracellular domain of recombinant CD123 were dependent on the protease activity of papain and bromelain. Pre-treatment of murine basophils with papain resulted in inhibition of IL-3-IL-3R signaling and suppressed IL-3- but not thymic stromal lymphopoietin-induced expansion of basophils in vitro. Our unexpected findings illuminate a novel mechanism for the regulation of basophil functions by protease antigens. Because IL-3 plays pivotal roles in the activation and proliferation of basophils and in protective immunity against helminth parasites, pathogen-derived proteases might contribute to the pathogenesis of infections by regulating IL-3-mediated functions in basophils.

1. Introduction

Th2 type immune responses play crucial roles in allergic disorders and in protective immunity against parasites. Helminth invasion induces Th2-related cytokines including IL-3, IL-4, IL-5, IL-9, IL-10, IL-13, IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), thereby mediating expanded populations of eosinophils, basophils, mast cells and other innate immune cell populations [1–3]. Basophils are basophilic granulocytes and represent <1% of peripheral blood leukocytes [4]. Basophils in humans and mice express FcεRI and readily generate large quantities of IL-4, in both an IgE-dependent and -independent manner, in response to various stimuli including parasite-derived antigens [5]. Recent studies have demonstrated the contribution of basophils to protection against both ecto- and endoparasites and to induction of Th2 type immune responses that are triggered after parasite infections [5]. Basophil numbers are increased in several animal models of helminth infections in an IL-3-dependent manner, and mice lacking IL-3 show delayed expulsion of certain helminths [6,7].

Helminth parasites secrete a variety of proteases, which play crucial roles in their virulence including in host entry, tissue migration and suppression of host immune effector cell functions [8]. Many allergens and allergen sources also have intrinsic protease activity [9]. A number of pathogen-derived antigens belong to the clan CA family (papain family) of cysteine proteases, as do allergens such as Der p 1 and Der f 1 from house dust mites and the papain from papaya (see MEROPS – [10]). Papain is a potent allergen, is associated with occupational allergy in humans [11],

Abbreviations: Ab, antibody; BMB, bone marrow-derived basophil; IL-3R, IL-3 receptor; LC-MS, liquid chromatography-mass spectrometry; MFI, mean fluorescence intensity; PAR, protease-activated receptor; rIL-3, recombinant IL-3; TSLP, thymic stromal lymphopoietin.

* Corresponding author. Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Fax: +81 3 3813 5512.
E-mail address: t-takai@juntendo.ac.jp (T. Takai).

http://dx.doi.org/10.1016/j.bbrc.2015.03.022

and can cause strong Th2 type immune responses [12–15] and allergic airway inflammation in mice [13,14,16,17]. However, although the link between protease activity and Th2 type immunity is well documented, the mechanisms through which host immune responses are regulated are not fully understood.

Papain induces cytokine/chemokine production in various types of cells, including in basophils, which is partially mediated by the protease-activated receptor 2 (PAR2) [18,19]. Protease allergens also modulate host immune cell functions indirectly via cleavage of immunoreceptors such as CD25 and CD23 [9,20–22]. We therefore considered that there must be target molecules other than PAR-2 on basophils that can be cleaved by papain. In the present study, we found that the cysteine protease activity of papain and bromelain selectively cleaves the α subunit of the IL-3 receptor (IL-3Rα/CD123) on the surface of murine basophils, resulting in inhibition rather than activation of IL-3-IL-3R signaling. Our unexpected findings illuminate a novel mechanism for the regulation of basophil functions by pathogen-derived protease antigens.

2. Materials and methods

2.1. Antibodies (Abs) and other reagents

Biotinylated anti-mouse CD49b (DX5), and PE-conjugated anti-mouse CD11b (M1/70) and CD131 (JORO50) were purchased from BD Pharmingen (San Diego, CA). FITC-conjugated anti-mouse FcεRIα (MAR-1) was from eBioscience (San Diego, CA). PE-conjugated anti-mouse CD45 (30-F11), CD69 (H1.2F3), CD123 (5B11), CD200R (OX-110), and allophycocyanin-conjugated streptavidin were from Biolegend (San Diego, CA). These Abs were used for flow cytometric analysis. Purified polyclonal anti-STAT5 and phosphorylated-STAT5 were purchased from Cell Signaling Technology (Tokyo, Japan) and were used for immunoblot analysis. Papain and bromelain were purchased from Calbiochem (San Diego, CA) and, when required, were heat-inactivated by 10-min incubation at 100 °C [12]. Protein concentrations were measured using the BCA Protein Assay Kit (Pierce, Rockford, IL). Murine recombinant IL-3 (rIL-3) and rTSLP were purchased from Wako Pure Chemicals (Osaka, Japan) and R&D Systems (Minneapolis, MN), respectively.

2.2. Mice, and bone marrow cultures

C57BL/6 mice (7–10 weeks old) were purchased from CLEA Japan (Tokyo, Japan). All animals were used in accordance with the guidelines of the institutional committee of Juntendo University. To obtain a basophil-enriched population, bone marrow cells prepared from the tibias and femurs of mice were cultured for 8 days in RPMI 1640 medium (Sigma–Aldrich, St. Louis, MO) supplemented with 2 mM L-glutamine, 0.05 mM 2-ME, 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.3 ng/ml rIL-3 [23], yielding a cell population in which $CD49b^{+}Fc\varepsilon RI\alpha^{+}$ bone marrow-derived basophils (BMBs) represented 50–60% of the cells. For flow cytometric analysis, total IL-3-cultured cells or freshly-isolated bone marrow cells were adjusted to a final concentration of 1×10^6 cells/ml and were incubated at 37 °C with papain or bromelain in supplemented RPMI medium without FCS and rIL-3. After incubation, the cells were washed and used for subsequent analysis. For ELISA analyses of CD123, culture supernatants of papain-treated cells were collected and further incubated for 40 min at 37 °C with 10 μM E64 (Peptide Institute, Osaka, Japan) to inhibit the cysteine protease activity of papain. For immunoblot analysis, IL-3-cultured bone marrow cells were incubated for 16 h without rIL-3 prior to stimulation. After cell stimulation, cells were washed twice with PBS and lysed with lysis buffer containing 50 mM Tris–HCl, 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 1% Nonidet P-40, protease inhibitor cocktail (Sigma–Aldrich), and phosphatase inhibitor cocktail (Sigma–Aldrich). Cell lysates were further treated with 10 μM E64 for 40 min at 37 °C before addition of sample buffer containing 2-mercaptoethanol for SDS-PAGE and heated for 3 min at 95 °C. For basophil counting, freshly isolated bone marrow cells were incubated for 1 h at 37 °C with 100 μg/ml papain or inactive papain in RPMI medium, washed, and cultured for 72 h in the presence of 0.3 ng/ml rIL-3 or 1 μg/ml rTSLP at a density of 250,000 cells/500 μl/well in 24-well plates. Non-adherent cells were collected and the number of live cells was counted by trypan blue exclusion. Basophils were identified as $CD49b^{+}Fc\varepsilon RI\alpha^{+}$ cells in flow cytometric analysis.

2.3. Flow cytometric analysis

Cells were pre-incubated with normal rat serum on ice for 15 min prior to incubation with the indicated combination of Abs, to prevent the nonspecific binding of irrelevant Abs. Stained cells were analyzed using a FACSCalibur (BD Biosciences, San Jose, CA), and data were analyzed with FlowJo software (Tree Star, Ashland, OR). Dead cells stained with propidium iodide were excluded from the analyses.

2.4. Cleavage of rIL-3R

For ELISA analysis, a murine recombinant IL-3Rα-human IgG Fc protein (rIL-3Rα) (R&D Systems) was incubated for 1 h at 37 °C with papain or bromelain in PBS. For liquid chromatography-mass spectrometry (LC-MS), 2 mg/ml rIL-3Rα was incubated for 1 h at 37 °C with 20 μg/ml papain in PBS. All samples were further incubated for 40 min at 37 °C with 10 μM E64 prior to subsequent analyses.

2.5. ELISA

For detection of IL-3Rα, 96-well plates were coated with monoclonal anti-mouse CD123 Ab (5B11, Biolegend) overnight at 4 °C (1/200 dilution, 50 μl/well), and were blocked with 20% (v/v) ImmunoBlock (DS Pharma Biomedical, Osaka, Japan) (60 μl/well). Bound IL-3Rα was detected with polyclonal goat anti-mouse IL-3Rα (AF983, R&D Systems) (0.5 μg/ml, 50 μl/well) followed by horseradish peroxidase (HRP)-conjugated donkey anti-goat IgG Ab (sc-2056, Santa Cruz Biotechnology, Santa Cruz, CA) (1/20000 dilution, 50 μl/well). HRP activity was detected by the color development for 20 min using tetramethyl benzidine (BD-OptEIA kit; BD-biosciences) and the reaction was stopped by adding sulfuric acid. The optical density at 450 nm, from which that at 570 nm was subtracted, was used as the signal.

2.6. Immunoblot analysis

Cell lysates prepared as described above were boiled in sample buffer, separated by SDS-PAGE and electrophoretically transferred to polyvinylidene difluoride membranes, followed by immunoblot analysis with various primary and HRP-labeled secondary Abs. Blots were developed with Luminata Forte Western HRP Substrate (Millipore, Bedford, MA) and were analyzed with the LAS-4000 (FujiFilm, Tokyo, Japan).

2.7. Liquid chromatography/mass spectrometry (LC–MS) analysis and identification of several protease-cleaved fragments of IL-3Rα

Murine rIL-3Rα (2 mg/ml) was incubated for 1 h at 37 °C with 20 μg/ml papain in PBS. After incubation, the samples were

treated with E64, diluted 100-fold with distilled water, and were then analyzed using LC-MS. The fragment peptides of IL-3Rα were analyzed using a nano-flow LC–MS system with a direct nano-flow LC system (DiNa; KYA Technologies, Hachioji, Japan) and an LTQ Orbitrap XL ETD mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). The samples were injected into a nano LC Column, C18 RP chromatograph (0.1 mm, inner diameter X 100 mm, KYA Technologies) and eluted with 0.1% formic acid (solvent A) and 0.1% formic acid in 90% CH3–CN (solvent B) using a program of 0% solvent B for 30 min, a gradient of 1.45%/min for 55 min, 100% solvent B for 10 min, and a flow rate of 300 nl/min. The eluted peptides were introduced online into the mass spectrometer. Data were searched against the UniPortKB/SwissProt database using the Mascot search engine (Matrix Science Inc, Boston, MA) [24,25].

2.8. Statistical analysis

The statistical significance of differences between groups was analyzed using Student's *t*-test (two-tailed). p values <0.05 were considered statistically significant.

3. Results

3.1. The cysteine proteases papain and bromelain cleave CD123, the α subunit of the IL-3R, on murine basophils

To identify target molecules for papain on basophils, we analyzed the effect of papain treatment of basophils on the expression of various cell surface markers using flow cytometry (Fig. 1). Unexpectedly, compared with control inactive papain-treated basophils, papain-treated basophils showed reduced expression of CD123, the α subunit of the IL-3R, while expression of other molecules such as CD11b, CD69, CD200R, CD131 (the common β subunit of IL-3, IL-5 and the GM-CSFR) and CD45 was unchanged or increased (Fig. 1B). Similar results were obtained in IL-3-cultured BMBs treated with papain (Fig. 1C) or with bromelain, a pineapple-derived cysteine protease (Fig. 1D). Treatment with papain or bromelain reduced the surface expression of CD123 on BMBs in a dose- and time-dependent manner (Fig. 2A–D) and the cysteine protease-specific inhibitor E64 abolished protease-induced cleavage of CD123 (Fig. 2E and F). The combined data indicate that both papain and bromelain cleave murine CD123 through their cysteine protease activity.

3.2. Papain and bromelain cause degradation of murine CD123

In previous reports, the house dust mite allergen Der p 1 was shown to cleave various cell surface immunoreceptors, thereby releasing soluble forms of some receptors into culture supernatants [9,21,26–28]. We next determined if ELISA assays could detect a cleaved fragment of CD123 in papain-treated supernatants. In our hands, approximately >0.6 ng/ml of a murine rIL-3Rα-Fc protein could be detected by ELISA (Fig. 3A). However, no CD123 fragment could be detected in the culture supernatants of BMBs incubated with papain (Fig. 3B), indicating that CD123 fragment concentration was below the detection limit, and/or that cleaved fragment(s) could not be captured by the Abs used in this system. Nevertheless, less murine rIL-3Rα-Fc protein was detectable by ELISA after incubation with papain or bromelain (Fig. 3C

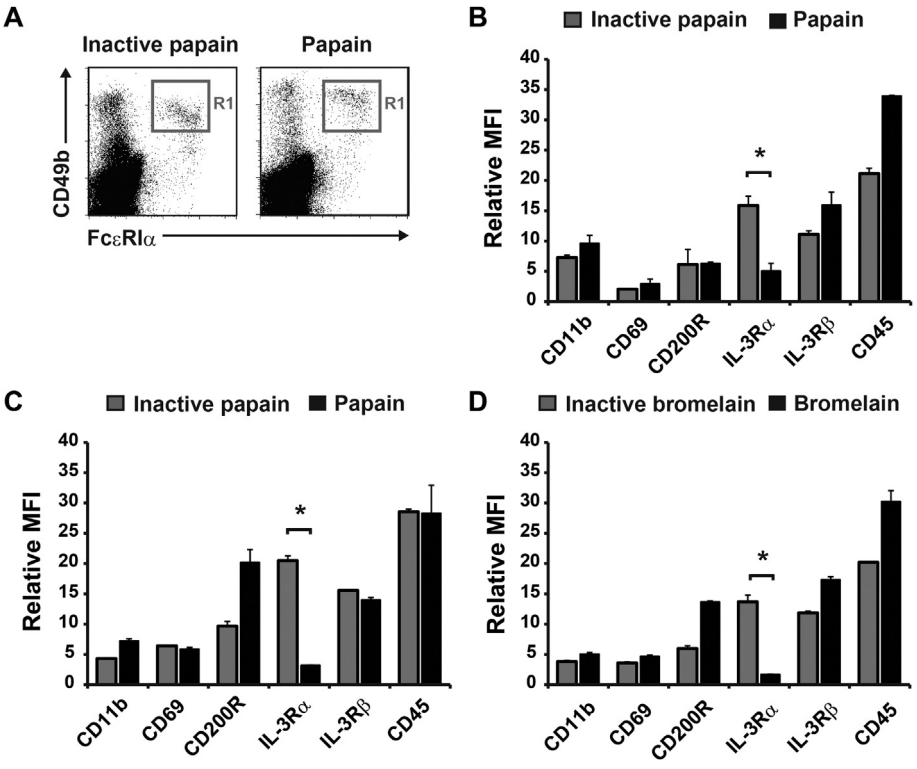


Fig. 1. The effect of the proteolytic activity of cysteine proteases on murine basophil surface markers. (**A**) Representative FACS staining profiles of CD49b and FcεRIα of freshly prepared bone marrow cells from C57BL/6 mice after incubation for 1 h at 37 °C with 100 μg/ml papain or heat-inactivated papain. (**B**) Relative mean fluorescence intensity (MFI) of the indicated markers (MFI of specific Ab/MFI of control isotype-matched Ab) gated on $CD49b^{+}FcεRIα^{+}$ basophils contained in the bone marrow cells. (*R1* gate in ***A***). (**C** and **D**) Relative MFI of the indicated markers gated on $CD49b^{+}FcεRIα^{+}$ basophils contained in IL-3-cultured bone marrow cells after incubation for 1 h with 100 μg/ml papain or heat-inactivated papain (***C***) or with 50 μg/ml bromelain or inactive bromelain (***D***). Data are indicated as means ± SD of duplicate cultures. $^{*}p < 0.05$ by a *t*-test. Data shown are representative of three independent experiments with similar results.

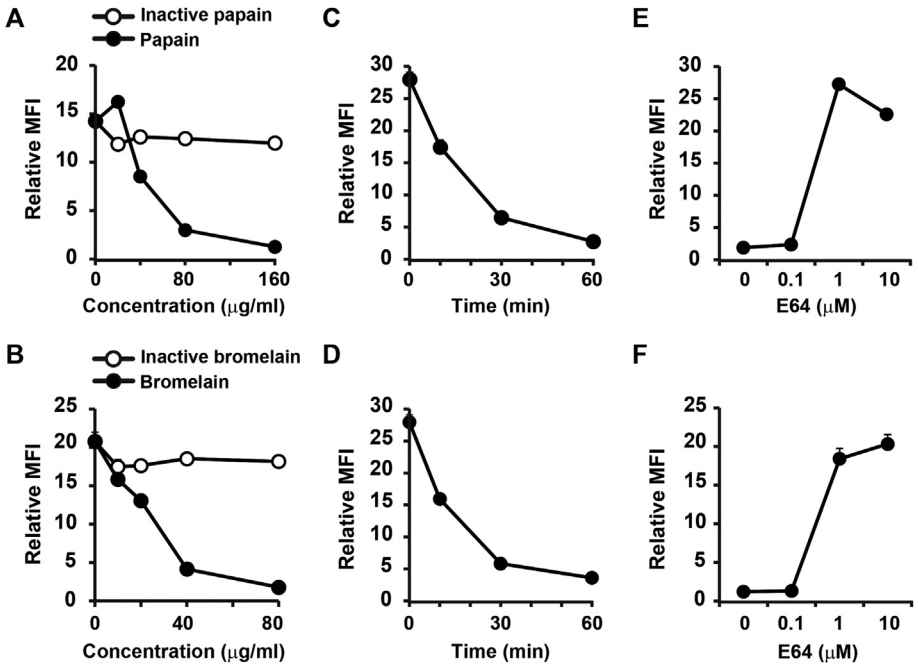
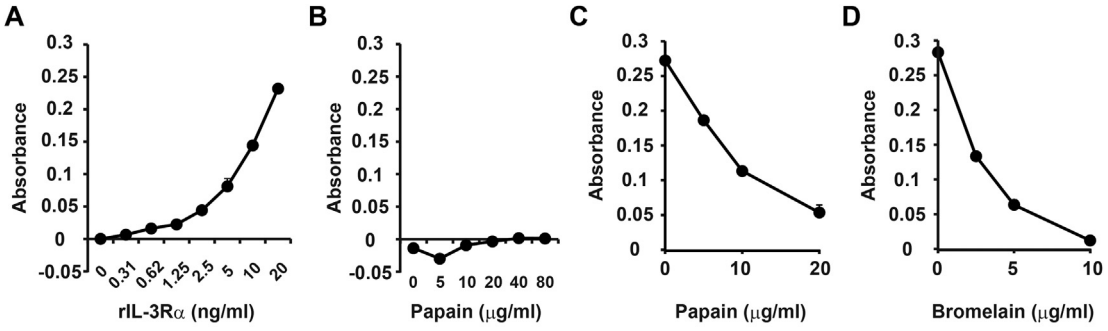


Fig. 2. Cysteine protease activity of papain and bromelain cleaves murine CD123 on IL-3-cultured BMBs. Dose dependency (1 h incubation) (**A** and **B**) and time dependency (**C** and **D**) of cysteine protease cleavage of murine CD123. (**E** and **F**) Effect of inhibition of protease activity by E64. Total IL-3-cultured bone marrow cells were incubated with papain, heat-inactivated papain or papain plus E64 (***A, C*** and ***E***) or with 50 μg/ml bromelain, heat-inactivated bromelain or bromelain plus E64 (***B, D*** and ***F***) and the relative MFI of CD123 on gated $CD49b^{+}Fc\varepsilon RI\alpha^{+}$ BMBs was analyzed. Concentrations of papain and bromelain were: as indicated (***A*** and ***B***), 100 μg/ml (***C*** and ***E***) or 50 μg/ml (***D*** and ***F***). Data are indicated as means ± SD of duplicate cultures. Data shown are representative of three independent experiments with similar results.

and D). Moreover several IL-3Rα fragments were detected by mass spectrometry analysis after papain treatment (Fig. 3E). These data indicate that papain and bromelain can cause the degradation of murine CD123.

3.3. Papain treatment suppresses IL-3-IL-3R signaling in basophils

We next questioned whether protease-mediated degradation of CD123 results in inhibition of IL-3-IL-3R signaling, or, alternatively,



E mouse IL-3Rα extra cellular domain (S17-K331)

SDLAAVREAPPTAVTTPIQNLHIDPAHYTLSWDPAPGADITTGAFCRKGR

DIFVWADPGLARCSFQSLSLCHVTNFTVFLGKDRAVAGSIQFPPDDDGDH

EAAAQDLRCWVHEGQLSCQWERGPKATGDVHYRMFWRDVRLGPAHNRECP

HYHSLDVNTAGPAPHGGHEGCTLDLDTVLGSTPNSPDLVPQVTITVNGSG

RAGPVPCMDNTVDLQRAEVLAPPTLTVECNGSEAHARWVARNRFHHGLLG

YTLQVNQSSRSEPQEYNVSIPHFWVPNAGAISFRVKSRSEVYPRKLSSWS

EAWGLVCPPEVMPVK

Fig. 3. Papain and bromelain can cause degradation of murine rIL-3Rα/CD123. (**A**) Detection of a murine recombinant IL-3Rα-human IgG Fc fusion protein (rIL-3Rα) by ELISA. (**B**) ELISA analysis of rIL-3Rα in the culture supernatant of total IL-3-cultured bone marrow cells after 1 h incubation with papain. (**C** and **D**) ELISA analysis of rIL-3Rα after 1 h incubation at 37 °C with papain (***C***) or bromelain (***D***). (**E**) LC-MS analysis or papain cleavage of rIL-3Rα. The amino acid sequence of the extracellular domain of the rIL-3Rα is shown. The cleaved fragments detected by LC-MS analysis are underlined. Data are indicated as means ± SD of duplicate cultures (***A-D***). Data shown are representative of three (***A-D***) or two (***E***) independent experiments with similar results.

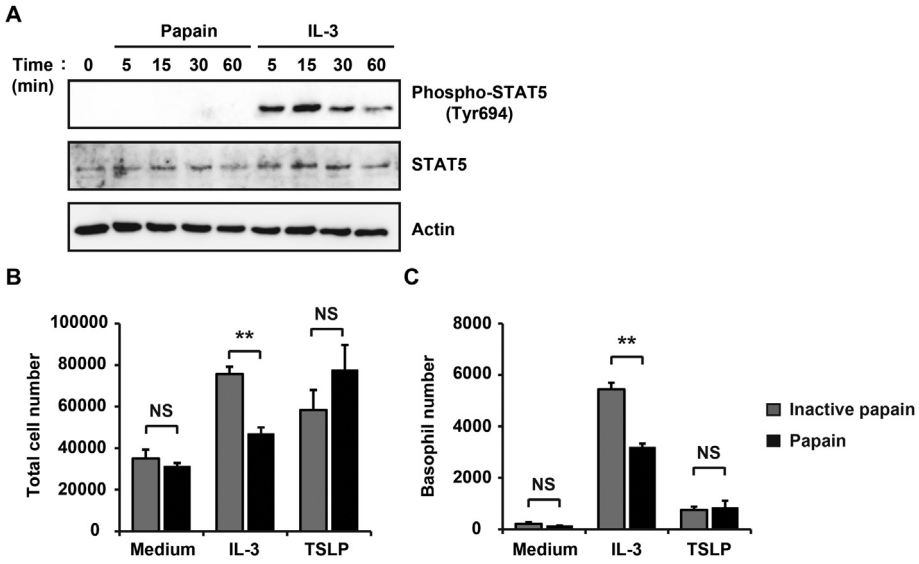


Fig. 4. Pretreatment with papain suppresses IL-3-IL-3R signaling in basophils. (**A**) Absence of STAT5 phosphorylation upon papain treatment. Total IL-3-cultured bone marrow cells were incubated for 16 h without rIL-3 and were then incubated with 100 μg/ml papain or 10 ng/ml rIL-3. Cells were collected at the indicated time points and whole cell lysates were prepared. Lysates were subjected to immunoblot analysis using Abs against the naive and phosphorylated form of STAT5 and against β-actin (loading control). (**B** and **C**) Total bone marrow cells were incubated with 100 μg/ml papain or with inactive papain at 37 °C. One hour later, the cells were washed and were then cultured for 72 h with 0.3 ng/ml rIL-3 or 1 μg/ml rTSLP, or without cytokines (Medium) at a density of 250,000 cells/well. The number of total bone marrow cells (***B***) or $CD49b^{+}Fc\varepsilon RI\alpha^{+}$ basophils (***C***) is shown (mean ± SD of triplicate cultures). Data shown are representative of three independent experiments with similar results. $^{**}p < 0.01$ by a *t*-test. NS: no significant difference.

triggers signal transduction downstream of the IL-3R. Although treatment with papain can reduce the surface expression of CD123 within 10 min as described above (Fig. 2C), tyrosine phosphorylation of STAT5, an immediate event induced by IL-3, was not observed in papain-treated BMBs (Fig. 4A). Instead, compared to control cells, bone marrow cells treated with papain showed lower responsiveness to IL-3, as judged by the number of cells after culture (Fig. 4B). Furthermore, pretreatment with papain also resulted in reduced IL-3 induction of basophil expansion, whereas it did not alter TSLP-induced expansion of basophils that occurs independently of IL-3 (Fig. 4C) [29]. These results suggest that papain treatment causes inhibition but not activation of IL-3-IL-3R signaling.

4. Discussion

Protease allergens modulate host immune effector cell functions directly and indirectly via proteolytic cleavage of various molecules including tight junction molecules, cell surface molecules, and endogenous protease inhibitors [9,20]. House dust mite-derived protease allergens can cleave surface molecules of PAR2, CD25, CD23, CD40, DC-SIGN, DC-SIGNR and IL-13Rα2 [9,20–22]. Recent studies identified PAR2 as a target for papain [18,19]; however, little is known about other receptors that might be cleaved by papain. We found that papain and bromelain can cause degradation of CD123, the murine IL-3R α subunit, which results in inhibition rather than activation of IL-3-mediated function in basophils.

IL-3 is a hematopoietic cytokine that stimulates the survival, proliferation and differentiation of hematopoietic progenitor cells [30] and is also a potent primer of basophils [31]. IL-3 plays crucial roles in protective immunity against certain parasite infections. Nematode *Strongyloides venezuelensis* and *Nippostrongylus brasiliensis* infection causes hyperplasia of tissue mast cells, and enhanced production of peripheral basophils [6,7]. Mice lacking IL-3 fail to develop mast cell/basophil populations and show delayed expulsion of worms in *S. venezuelensis* infection [7]. Moreover, basophil ablation leads to impaired expulsion of *N. brasiliensis* in a model of secondary infection [32]. Furthermore, exogenous IL-3 injection promotes worm expulsion in mice infected with the nematode *Trichinella spiralis* [33]. Thus, protease-mediated cleavage of the IL-3R could be a possible strategy for certain pathogens for suppression of host immune effector systems. In our preliminary flow cytometric analysis, papain and bromelain failed to cleave human CD123 (data not shown). This result might be associated with the low homology between mouse and human CD123 (30% identity) [34]. Considering the importance of IL-3 in immunity against parasites, it is likely that during evolution the amino acid residues of CD123 changed, resulting in a human CD123 that could avoid the cleavage caused by pathogen-derived proteases. However, we cannot exclude the possibility that papain-like cysteine proteases other than those used in this study can cleave human CD123.

Human malaria *Plasmodium falciparum* also expresses a number of papain-family cysteine proteases, including falcipains, and their homologues have also been identified in other plasmodial species [35]. Malaria infection causes transient induction of IL-3 in both humans and mice, and basophil number is increased in a model of mouse malaria [36–38]. The role of basophils in protection against malaria parasites still remains to be elucidated. However, the non-synonymous polymorphism of the *IL3* gene is associated with the protection of recurrent malaria attacks in African populations [39]. Future studies may uncover yet unrecognized roles of IL-3 and cysteine protease antigens in the pathophysiology of malaria.

In conclusion, the current study identified IL-3R as a novel target of papain-family cysteine proteases. The drug potency of parasite clan CA cysteine protease inhibitors is well established [40–42]. Our findings might facilitate the understanding of host-pathogen interactions, and also cast new light on the development of new anti-parasitic chemotherapy.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank Seiji Kamijo for comments and technical advice and Michiyo Matsumoto for secretarial assistance. This study was supported by Ministry of Education, Culture, Sports, Science and Technology (MEXT)-supported Program for Strategic Research Foundation at Private Universities (S1101010).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.022.

References

[1] J.E. Allen, R.M. Maizels, Diversity and dialogue in immunity to helminths, Nat. Rev. Immunol. 11 (2011) 375–388.
[2] J.G. Perrigoue, F.A. Marshall, D. Artis, On the hunt for helminths: innate immune cells in the recognition and response to helminth parasites, Cell. Microbiol. 10 (2008) 1757–1764.
[3] S.F. Ziegler, D. Artis, Sensing the outside world: TSLP regulates barrier immunity, Nat. Immunol. 11 (2010) 289–293.
[4] S.J. Galli, Mast cells and basophils, Curr. Opin. Hematol. 7 (2000) 32–39.
[5] H. Karasuyama, T. Wada, S. Yoshikawa, et al., Emerging roles of basophils in protective immunity against parasites, Trends Immunol. 32 (2011) 125–130.
[6] C.S. Lantz, B. Min, M. Tsai, et al., IL-3 is required for increases in blood basophils in nematode infection in mice and can enhance IgE-dependent IL-4 production by basophils in vitro, Lab. Invest 88 (2008) 1134–1142.
[7] C.S. Lantz, J. Boesiger, C.H. Song, et al., Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites, Nature 392 (1998) 90–93.
[8] M.W. Robinson, J.P. Dalton, S. Donnelly, Helminth pathogen cathepsin proteases: it's a family affair, Trends Biochem. Sci. 33 (2008) 601–608.
[9] T. Takai, S. Ikeda, Barrier dysfunction caused by environmental proteases in the pathogenesis of allergic diseases, Allergol. Int. 60 (2011) 25–35.
[10] N.D. Rawlings, A.J. Barrett, A. Bateman, MEROPS: the database of proteolytic enzymes, their substrates and inhibitors, Nucleic Acids Res. 40 (2012) D343–D350.
[11] H.S. Novey, L.E. Marchioli, W.N. Sokol, et al., Papain-induced asthma–physiological and immunological features, J. Allergy Clin. Immunol. 63 (1979) 98–103.
[12] C.L. Sokol, G.M. Barton, A.G. Farr, et al., A mechanism for the initiation of allergen-induced T helper type 2 responses, Nat. Immunol. 9 (2008) 310–318.
[13] S. Kamijo, H. Takeda, T. Tokura, et al., IL-33-mediated innate response and adaptive immune cells contribute to maximum responses of protease allergen-induced allergic airway inflammation, J. Immunol. 190 (2013) 4489–4499.
[14] T.Y. Halim, C.A. Steer, L. Matha, et al., Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation, Immunity 40 (2014) 425–435.
[15] H. Iida, T. Takai, Y. Hirasawa, et al., Epicutaneous administration of papain induces IgE and IgG responses in a cysteine protease activity-dependent manner, Allergol. Int. 63 (2014) 219–226.
[16] K. Oboki, T. Ohno, N. Kajiwara, et al., IL-33 is a crucial amplifier of innate rather than acquired immunity, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 18581–18586.
[17] T.Y. Halim, R.H. Krauss, A.C. Sun, et al., Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation, Immunity 36 (2012) 451–463.
[18] H. Kouzaki, S.M. O'Grady, C.B. Lawrence, et al., Proteases induce production of thymic stromal lymphopoietin by airway epithelial cells through protease-activated receptor-2, J. Immunol. 183 (2009) 1427–1434.
[19] G. Liang, T. Barker, Z. Xie, et al., Naive T cells sense the cysteine protease allergen papain through protease-activated receptor 2 and propel TH2 immunity, J. Allergy Clin. Immunol. 129 (2012) 1377–1386 e13.
[20] F. Shakib, A.M. Ghaemmaghami, H.F. Sewell, The molecular basis of allergenicity, Trends Immunol. 29 (2008) 633–642.
[21] T. Takai, Mite endopeptidase 1, in: N.D. Rawlings, G.S. Salvesen (Eds.), Handbook of Proteolytic Enzymes, Academic Press, Oxford, 2013, pp. 1957–1963.
[22] T. Takai, Serine endopeptidase allergens from *Dermatophagoides* species, in: N.D. Rawlings, G.S. Salvesen (Eds.), Handbook of Proteolytic Enzymes, Academic Press, Oxford, 2013, pp. 3055–3060.
[23] C. Ohnmacht, D. Voehringer, Basophil effector function and homeostasis during helminth infection, Blood 113 (2009) 2816–2825.
[24] T. Fujimura, Y. Shinohara, B. Tissot, et al., Glycosylation status of haptoglobin in sera of patients with prostate cancer vs. benign prostate disease or normal subjects, Int. J. Cancer 122 (2008) 39–49.
[25] E. Yamamoto, T. Uchida, H. Abe, et al., Increased expression of ERp57/GRP58 is protective against pancreatic beta cell death caused by autophagic failure, Biochem. Biophys. Res. Commun. 453 (2014) 19–24.
[26] O. Schulz, H.F. Sewell, F. Shakib, Proteolytic cleavage of CD25, the alpha subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity, J. Exp. Med. 187 (1998) 271–275.
[27] A.M. Ghaemmaghami, L. Gough, H.F. Sewell, et al., The proteolytic activity of the major dust mite allergen Der p 1 conditions dendritic cells to produce less interleukin-12: allergen-induced Th2 bias determined at the dendritic cell level, Clin. Exp. Allergy 32 (2002) 1468–1475.
[28] M.O. Daines, W. Chen, Y. Tabata, et al., Allergen-dependent solubilization of IL-13 receptor alpha2 reveals a novel mechanism to regulate allergy, J. Allergy Clin. Immunol. 119 (2007) 375–383.
[29] M.C. Siracusa, S.A. Saenz, D.A. Hill, et al., TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation, Nature 477 (2011) 229–233.
[30] J.N. Ihle, Interleukin-3 and hematopoiesis, Chem. Immunol. 51 (1992) 65–106.
[31] P. Valent, G. Schmidt, J. Besemer, et al., Interleukin-3 is a differentiation factor for human basophils, Blood 73 (1989) 1763–1769.
[32] C. Ohnmacht, C. Schwartz, M. Panzer, et al., Basophils orchestrate chronic allergic dermatitis and protective immunity against helminths, Immunity 33 (2010) 364–374.
[33] M. Korenaga, T. Abe, Y. Hashiguchi, Injection of recombinant interleukin 3 hastens worm expulsion in mice infected with Trichinella spiralis, Parasitol. Res. 82 (1996) 108–113.
[34] T. Hara, A. Miyajima, Two distinct functional high affinity receptors for mouse interleukin-3 (IL-3), EMBO J. 11 (1992) 1875–1884.
[35] P.J. Rosenthal, Cysteine proteases of malaria parasites, Int. J. Parasitol. 34 (2004) 1489–1499.
[36] H. Burgmann, S. Looareesuwan, E.C. Wiesinger, et al., Levels of stem cell factor and interleukin-3 in serum in acute Plasmodium falciparum malaria, Clin. Diagn Lab. Immunol. 4 (1997) 226–228.
[37] H. Helmby, M. Kullberg, M. Troye-Blomberg, Expansion of IL-3-responsive IL-4-producing non-B non-T cells correlates with anemia and IL-3 production in mice infected with blood-stage Plasmodium chabaudi malaria, Eur. J. Immunol. 28 (1998) 2559–2570.
[38] M. Poorafshar, H. Helmby, M. Troye-Blomberg, et al., 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 (2000) 2660–2668.
[39] C.G. Meyer, M.H. Calixto Fernandes, C.D. Intemann, et al., IL3 variant on chromosomal region 5q31-33 and protection from recurrent malaria attacks, Hum. Mol. Genet. 20 (2011) 1173–1181.
[40] A.R. Renslo, J.H. McKerrow, Drug discovery and development for neglected parasitic diseases, Nat. Chem. Biol. 2 (2006) 701–710.
[41] J.C. Engel, P.S. Doyle, I. Hsieh, et al., Cysteine protease inhibitors cure an experimental Trypanosoma cruzi infection, J. Exp. Med. 188 (1998) 725–734.
[42] M.H. Abdulla, K.C. Lim, M. Sajid, et al., Schistosomiasis mansoni: novel chemotherapy using a cysteine protease inhibitor, PLoS Med. 4 (2007) e14.