Mast cell-derived serine proteinase regulates T helper 2 polarization

Zhi-Qiang Liu1,4*, Jiang-Ping Song3*, Xiaoyu Liu1*, Jing Jiang1,4*, Xiao Chen3, Liao Yang4, Tianyong Hu4, Peng-Yuan Zheng2, Zhi-Gang Liu1 & Ping-Chang Yang1

1ENT Institute of Shenzhen University, State Key Laboratory of Respiratory Disease for Allergy at Shenzhen University, Shenzhen Key Laboratory of Allergy & Immunology, Shenzhen University School of Medicine, Shenzhen, China, 2Department of Gastroenterology, Zhengzhou University, Zhengzhou, China, 3State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, 4Longgang Central Hospital, ENT Hospital, Shenzhen ENT Institute, Shenzhen, China.

Although mast cells play a critical role in allergic reactions, the cells are also involved in the protective immunity in the body. This study aims to investigate the role of mast cells in immune regulation during aberrant T helper (Th)2 responses. In this study, an adoptive antigen-specific Th2 response model was established with mast cell-deficient mice to test the role of mast cell in the immune regulation. Cell culture was employed to test the role of mast cells in the modulation of the expression of B cell lymphoma 6 protein (Bcl-6) in Th2 cells. The results showed that after adoptive transfer with immune cells, the mast cell-deficient mice showed stronger Th2 pattern responses in the intestine than that in the mast cell-sufficient mice. Mast cell-derived mouse mast cell protease-6 increased the expression of Bcl-6 in Th2 cells. Bcl-6 inhibited the expression of GATA-3 in Th2 cells, subsequently, forkhead box P3 was increased and the Th2 cytokines were reduced in the cells; the cells thus showed the immune regulatory properties similar to regulatory T cells. We conclude that besides initiating immune inflammation, mast cells also contribute to the immune regulation on Th2 polarization.

Besides functioning as effector cells in the initiation of the immediate allergic reactions, mast cells are also involved in the adaptive immunity. Mast cells play a critical role in the establishment of the organ or tissue transplantation tolerance. Yet, whether mast cells play a role in regulation of the Th2 response is unknown.

Among the mediators of mast cells, the serine proteases, including tryptase in human mast cells, rat mast cell protease and mouse mast cell protease-6 (mMCP-6), have a strong immune regulatory capacity. The serine proteases activate the protease-activated receptors (PAR)1 and PAR2 to modulate the activities of target cells. During immune responses, mast cells may communicate with other immune cells, such as Th2 cells, on the sites. These immune cells thus have the opportunity to interact with each other. Th2 cells express PAR2, and the B-cell lymphoma 6 protein (Bcl-6); the latter can be regulated in the processes of Th2 responses. Whether mast cell-derived serine protease modulates the expression of Bcl-6 in Th2 cells is unclear.

In line with previous studies, we also found that the Th2 cells expressed Bcl-6 and PAR2; the latter could be activated by the mast cell-derived mMCP-6. The expression of Bcl-6 suppressed the expression of Th2 cytokines and increased the expression of forkhead box P3 (Foxp3) genes in Th2 cells, which contributed to the regulation of the skewed Th2 responses.

Results
Adaptive Th2 response is stronger in mast cell-deficient mice than in wild type littermates. Apart from being the major effector cells in allergic reactions, mast cells also play a role in immune tolerance, which implies that mast cells may be able to regulate the abnormal immune responses. To test the hypothesis, we adoptively transferred OVA-specific CD4+ CD25− T cells (10⁶ cells/mouse, from OTII mice; labelled with carboxyfluorescein succinimimidyl ester; CFSE) to mast cell-deficient KitW−/KitW− (W−/−) Mice. The mice were also adoptively transferred with saline, or reconstituted with OVA-specific IgE-sensitized bone marrow derived mast cells (Fig. S1, S2 in supplemental materials), or naive mast cells. The mice were then fed with OVA (the specific antigen; or fed with BSA using as a control) daily for 3 days and sacrificed on day 4. The lamina propria mononuclear cells (LPMC) were isolated from the small intestine and analyzed by flow cytometry. The CFSE-
Figure 1 | Mast cells trigger the immune regulatory response. CD4\(^+\) CD25\(^-\) T cells (isolated from OTII mice) were labelled with CFSE, and adoptively transferred to mast cell-deficient W\(^{−/−}\) mice (5 x 10\(^6\) cells/mouse). The mice were reconstituted with OVA-specific IgE-sensitized BMSCs, or naive BMSC. Also, the mice were fed with OVA (5 mg/mouse) or BSA daily for 3 days and sacrificed on day 4. The LPMCs were isolated and analyzed by flow cytometry. (A), the gated dot plots indicate the CFSE-labeled CD4\(^+\) T cells. (B–E), the histograms indicate the proliferating CD4\(^+\) T cells in the gated cells of (A), (F), the bars indicate the summarized data of (B–E). (G–J), the histograms indicate the frequency of IL-4\(^-\) T cells in the gated cells of (A), (K), the bars indicate the summarized data of (G–J). (L), the bars indicate the levels of IL-4 and IFN-\(\gamma\) in the culture supernatant (assessed by ELISA). * p < 0.01, compared with group E (F) or J (K, L). The data represent 3 separate experiments.

**Activation of mast cells increases the expression of Bcl-6 in CD4\(^+\) T cells.** We next cultured the OVA-specific IgE-sensitized BMSCs and OVA-specific CD4\(^+\) T cells in the presence of OVA and dendritic cells (DC). The CD4\(^+\) T cells were then isolated by magnetic cell sorting (MACS) and assessed the expression of Bcl-6. The results showed that the expression of Bcl-6 was detectable in non-stimulated CD4\(^+\) T cells; the activation of mast cells significantly increased the expression of Bcl-6 in the CD4\(^+\) T cells (Fig. 2A–B). The results were further confirmed by treating CD4\(^+\) T cells with recombinant mMCP-6 (Fig. 2C–D). To identify the mediators of mast cells that were involved in the mast cell-induced Bcl-6 expression in CD4\(^+\) T cells, we treated the BMSCs with five different mast cell mediator receptor antagonists, indomethacin (to inhibit prostaglandins) or zileuton (to inhibit the biosynthesis of leukotrienes) respectively in addition to the above procedures. The results showed that these antagonists did not show detectable inhibitory effects on the mast cell-induced Bcl-6 expression in CD4\(^+\) T cells (Fig. S4, Table S1 in supplemental materials).

**The autocrine Bcl-6 suppresses the expression of IL-4 in activated CD4\(^+\) T cells.** We also investigated if the activation of mast cells suppressed the Th2 responses. OVA-specific CD4\(^+\) T cells were isolated from the OTII mouse spleen. BMSCs (sensitized by OVA-specific IgE) were cultured with the CD4\(^+\) T cells in the presence of the specific Ag (OVA; to activate the sensitized mast cells) and DCs. The results showed that, compared with the non-activated BMSCs, the activated BMSCs significantly suppressed the Th2 responses, in which the frequency of IL-4\(^-\) (Fig. 3A, C) and IL-13\(^-\) (Fig. 3B, C) T cells, and the levels of IL-4 and IL-13 in the culture supernatants (Fig. 3D) were markedly suppressed, in spite of a certain amount of IL-4 and IL-13 might be released from the activated mast cells (Fig. 3D). The suppressive effect on Th2 response was abolished in the presence of anti-mMCP-6 antibody (Fig. 3A–D). The results indicate that mast cell-derived mMCP-6 suppresses Th2 response. To strengthen the results, we treated the OVA-specific CD4\(^+\) T cells with both OVA (in the presence of DC) and mMCP-6 directly in the culture. Indeed, the Th2 response did not occur (Fig. 3A–D). To clarify if the cell-cell contact is required in the
mast cell-suppressed Th2 response, in the same experimental procedures above, we separate mast cells and T cells in Transwell system. The results showed that the activation of mast cells still suppressed Th2 response (Fig. 3A–D).

Activated mast cells prevent antigen specific CD4+ T cells from activation-induced apoptosis and convert the antigen specific CD4+ T cells to Tregs. In general, after activation, the effector T cells become apoptotic, a phenomenon called the “activation induced cell death”. In the presence of specific IgE sensitized mast cells, whether the specific antigen-activated Th2 cells become apoptotic or differentiate into other cell types is unclear. We then adoptively transferred the OVA-specific T cells (labeled with CFSE) from OVA-sensitized BMMC into naive OTII mice (reconstituted with OVA specific IgE-sensitized mast cells). The mice were challenged with OVA daily for one week. The mice were sacrificed, LPMCs were analyzed by flow cytometry. The data showed that, cultured with medium alone showed 4.53% T cell proliferation (Fig. 6A, 6G); in the presence of anti-TGF-β antibody (Fig. 6D, 6G), but not by an anti-IL-10 antibody (Fig. 6E, 6G). The levels of Th2 cytokines, including IL-4, IL-5 and IL-13, were also changed in the culture supernatant in parallel with the changes of the effector T cell proliferation.

Figure 2 | Mast cells up-regulate the Bcl-6 expression in Th2 cells. IgE-sensitized BMMC were cultured with CD4+ T cells (isolated from OTII mouse spleen; 106 cells/ml; BMMC; T cell = 1: 10) in the presence of specific antigen OVA and DC (T cell: DC = 10: 1) for 0–6 h with the following conditions: (1) with or without the addition of anti-mMCP-6 antibody (Ab) [isotype IgG (cAb) was used as a control]. (2) CD4+ T cells were knocked down the PAR2 gene by PAR2 RNAi (shRNA); control shRNA (shCon) was used as a control. (3) In some experiments, mast cell mediator antagonists (C and D; see Table S1 for detail) were added to the culture. (4) The CD4+ T cells were treated with PAR2 active peptides (AP) (or control peptides; CP), (5) CD4+ T cells were treated with BSA and DC. (6) CD4+ T cells were cultured alone (T cell). The CD4+ T cells were isolated by MACS and processed for qRT-PCR and Western blotting or ELISA. (A), the bars indicate the Bcl-6 mRNA levels. (B), the immune blots indicate the Bcl-6 protein levels. The bars below the blots indicate the summarized integrated density of the immune blots. (C–D), OTII mice were fed with OVA (5 mg/mouse; using BSA as a control) for 48 h. OVA-specific CD4+ T cells were isolated from OTII mouse spleen by MACS and cultured in the presence of recombinant mMCP-6 at the indicated doses for 6 days. The cells were collected, RNA and protein were extracted and analyzed by qRT-PCR and Western blotting (D). The bars indicate the levels of Bcl-6 at mRNA level (C) and protein level (D). *, p < 0.01, compared with group “0 h” in (A–B), or the dose “0” group in (C–D). The data were presented as mean ± SD from 3 experiments.

Bcl-6 represses expression of GATA3 and promotes expression of Foxp3 in Th2 cells. Published data indicate that GATA3 directly binds to the Foxp3 promoter to suppress the transcription of the Foxp3 gene. The present data suggest that the increase in Bcl-6 may be involved in the conversion of antigen specific CD4+ T cells to Tregs. Based on the above information, we inferred that the expression of Bcl-6 repressed the activities of GATA3 in Th2 cells and resulted in the elevation of the Foxp3 expression in Th2 cells; the cells then differentiate into Foxp3+. To test the hypothesis, we exposed the polarized Th2 cells to mMCP-6 for 48 h, which resulted in marked suppression of GATA3 and increase in Foxp3 in the Th2 cells, but not in those cells with Bcl-6 gene silence; the results were further confirmed by the over expression of Bcl-6 in Th2 cells (Fig. 5; Fig. S6; Fig. S7).

To clarify that the induced Tregs had the immune suppressor function, we prepared Tregs and CD4+ CD25+ T cells (isolated from the OTII mouse spleen; labeled with CFSE; using as effector T cells). The two cell populations (in the presence of DC) were cultured in the presence of anti-CD3 and anti-CD28 for 3 days. The cells were analyzed by flow cytometry. The data showed that, cultured with medium alone showed 4.53% T cell proliferation (Fig. 6A, 6G); in the absence of Tregs, the OVA-specific CD4+ T cell markedly proliferated (43.6%, Fig. 6B, 6G), which was markedly suppressed by the presence of Tregs (5.10%, Fig. 6C, 6G), but abolished by the addition of anti-TGF-β antibody (Fig. 6D, 6G), but not by an anti-IL-10 antibody (Fig. 6E, 6G).
The results indicate that the induced Tregs have the immune suppressor function.

**Discussion**

Mast cells express IgE receptors, which bind IgE to form complexes on the surface of mast cells. Re-exposure to specific antigens results in forming complexes of specific antigen/specific IgE/FcεRI to activate mast cells, leading to releasing a number of chemical mediators to induce or strengthen immune inflammation. On the other hand, mast cells are also involved in the development of adaptive immunity. The present data add novel information to mast cells' properties by showing that mast cells induce the intestinal Th2 cells to express...
Bcl-6. The expression of Bcl-6 suppresses the expression of Th2 cytokines in Th2 cells and inhibits proliferation of Th2 cells. Such a function of mast cells is somewhat similar to the immune suppressor function of Tregs. Thus, in addition to those well-described functions in the initiation of allergy and inflammation, mast cells are also involved in the immune regulation. The phenomenon has been noted by investigators in the studies of the transplantation tolerance, in which mast cells play a pivotal role in the prevention of transplantation rejection.

We appreciate that under physiological environment, Th2 cells and their cytokines also present in the body; the cytokines play important roles in the immunity. The responses of Th2 cells are tightly regulated by the immune regulatory system in the body and under control in general. The Treg’s activities are proposed to be a critical factor in regulating the Th2 responses to avoid injuring the self-tissue. The present data pinpoint that the activated mast cells can initiate the immune regulatory activities by inducing the expression of Bcl-6 in Th2 cells. The regulatory process can be mediated by releasing the serine protease based on the evidence that (i) activated mast cells increased the expression of Bcl-6 in Th2 cells; (ii) pretreatment with anti-mMCP-6 antibody blocked the increase in Bcl-6 in Th2 cells. Published data indicate that mast cell-derived serine proteases can activate target cells via cleaving the PAR2; our data have expanded this notion with that (i) the knockdown of the gene of PAR2 inhibited the increase in the expression of Bcl-6 in Th2 cells induced by mast cells; (ii) exposure to PAR2 active peptide also induced the expression of Bcl-6 in Th2 cells.

Upon activation, the PAR2 induces MAP kinases phosphorylation to modulate the bioactivities of target cells; such as the treatment with active PAR2 peptides can increase the phosphorylation of MAPK/ERK kinases that also can be induced by mast cell activation; the activation of PAR2 increases intracellular reactive oxygen species also via the MAPK pathway. A similar phenomenon was noted in the present study; the results show that mast cell activation induced the increase of phosphorylated p38 MAPK and ERK1/2 in Th2 cells, which could be abrogated by pretreatment with the antagonists of p38 MAPK and ERK1/2. The downstream of PAR2 activation is related to gene transcription. Studying with 2500 human genes, Suen et al observed that PAR2 activation was associated with cellular metabolism genes, the cell cycle, the MAPK pathway, sirtuin enzymes, inflammatory cytokines, and anti-complement function. In line with these studies, the present data show that the activation of PAR2 can also induce the expression of Bcl-6 in Th2 cells.

Figure 5 | mMCP-6 modulate the expression of GATA3 and Foxp3 in Th2 cells. From the OTII mouse spleen, we prepared naïve CD4+ T cells and polarized Th2 cells. The polarized Th2 cells (10^6 cells/ml) were cultured in the presence of mMCP-6 (50 μM) for 48 h as denoted on the x axis. The cellular extracts were analyzed by qRT-PCR and Western blotting. (A–B), the bars indicate the mRNA levels of GATA3 (A) and Foxp3 (B). (C–D), the immune blots indicate the contents of protein of GATA3 (C) and Foxp3 (D). The bars below the immune blots show the summarized integrated density of the blots. The data in bar graphs were presented as mean ± SD from 3 experiments. Naive: Naïve CD4+ T cells. Medium: Cells were cultured with medium alone. mMCP6: Cells were cultured in the presence of mMCP-6. $: The CD4+ T cells were Bcl-6 deficient. Bcl6-Oex: Polarized OTII Th2 cells were generated using a Bcl-6 expressing plasmid to make the cells over express Bcl-6. *, p < 0.01, compared with naïve T cell group.
The immune responses, such as Th2 response, are originally protective in the body. Upon exposure to foreign antigen stimulation, Th0 cells differentiate into Th2 cells to produce IL-4, the latter further facilitate the production of antigen specific antibodies to establish the humoral immunity to specific antigens. However, over-productions of IL-4 and specific antibodies, such as IgE, induces a series of disorders, such as asthma, food allergy, etc. Therefore, to maintain the homeostasis, the body should have a mechanism to regulate the immune responses to an optimal range.

Apart from the mechanism of activation induced cell death, the Tregs can fulfill the task to regulate the ongoing immune reactions. Our data reveal a novel aspect of the Th2 response, some Th2 cells can be converted to Tregs by the activated mast cells; these Tregs have the suppressor function to suppress other effector T cell activities. The data further support the first experiment, in which the Th2 responses are more severe in the absence of the sensitized mast cells.

Induction of Tregs has been well investigated. A number of molecules, such as TGF-β, IL-10, epithelial cell-derived TGF-β, are recognized in the generation of Tregs. One of the key molecules in the induction of Tregs is the expression of Foxp3. In line with previous studies, we observed that the naïve CD4+ T cells (Th0 cells) expressed Foxp3 at very low or undetectable levels, which was up-regulated in Th2 cells by exposing to the activated-mast cells. Under physiological conditions, the GATA3 binds to Foxp3 promoter to prevent its transcription, thus to prevent Th0 cells from developing to Tregs. Upon the exposure to the activated mast cells, the expression of GATA3 was inhibited, which did not occur in Bcl-6-deficient Th2 cells. Others also noted that Bcl-6 can repress GATA3 in Th2 cells. The data support the inference of Bcl-6 repressing the expression of GATA3 in Th2 cells.

Using mMCP-6 deficient mice, others shows that mMCP-6 acts a proinflammatory mediator to recruit neutrophils to protect against infections. Shin et al observed that mast cell-derived mMCP-6 was a critical molecule in recruiting eosinophils to expel parasites from the infected sites. Our results demonstrate that mMCP-6 is also involved in the immune regulation. These data implicate that mast cell-derived mMCP-6 has an important role in the immune function in the body. The limitation of this study is that we have to use mast cell deficient mice to test our hypothesis. c-Kit is critically involved in the development and function of some stem cells and mature cells; these immune cells unrelated to the mast cell deficiency of c-Kit mutants may contribute to the outcome of experiments. Moreover, others have noted that in contrast c-Kit-dependent mast cell deficient mice, c-Kit-independent mast cell deficient mice showed a surprisingly normal immune system. Therefore, further investigations on this topic is necessary.

In summary, the present data demonstrate that activated mast cells increase the expression of Bcl-6 in Th2 cells to convert the Th2 cells to Tregs; the induced Tregs have the capability to suppress Th2 responses.

Methods

Ethic statement. The experimental procedures in this study were approved by the Animal Care Committee at Shenzhen University. The experimental procedures were presented in supplemental materials.

1. Galli, S. J., Nakae, S. & Tsai, M. Mast cells in the development of adaptive immune responses. Nat Immunol 6, 135–142 (2005).
2. de Vries, V. C., Elgueta, R., Lee, D. M. & Noelle, R. J. Mast Cell Protease 6 Is Required for Allograft Tolerance. Transplant Proc 42, 2759–2762 (2010).
3. Nakano, T. et al. Immunological and Regenerative Aspects of Hepatic Mast Cells in Liver Allograft Rejection and Tolerance. PLoS One 7, e37202 (2012).
4. Okonomopoulou, K. et al. Proteinase-mediated cell signalling: targeting proteinase-activated receptors (PARs) by kallikreins and more. Biol Chem 387, 677–685 (2006).
5. Mari, B. et al. Thrombin and trypsin-induced Ca(2+) mobilization in human T cell lines through interaction with different protease-activated receptors. FASEB J 10, 309–316 (1996).
6. Crotty, S., Johnston, R. J. & Schoenberger, S. P. Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. Nat Immunol 11, 114–120 (2010).
7. Takahashi, H. et al. TGF-[beta] and retinoic acid induce the microRNA miR-10a, which targets Bcl-6 and constrains the plasticity of helper T cells. Nat Immunol 13, 587–595 (2012).
8. Mantel, P. Y. et al. GATA3-driven Th2 responses inhibit TGF-beta1-induced Foxp3 expression and the formation of regulatory T cells. PLoS Biol 5, e329 (2007).
9. Kurugi, H. & Rajalingam, K. A Role for Prohibitin in Mast Cell Activation: Location Matters. Sci. Signal. 6, e29 (2013).
10. Jacob, C. et al. Mast Cell Tryptase Controls Paracutaneous Permeability of the Intestine. J Biol Chem 280, 31936–31948 (2005).
11. Gao, G., Ouyang, A., Kaufman, M. P. & Yu, S. ERK1/2 signaling pathway in mast cell activation-induced sensitization of esophageal nodose C-fiber neurons. Dis Esophagus 24, 194–203 (2011).
12. Banfi, C. et al. Mitochondrial reactive oxygen species: a common pathway for PAR1- and PAR2-mediated tissue factor induction in human endothelial cells. J Thromb Haemost 7, 206–216 (2009).

1. Banfi, C. et al. Mitochondrial reactive oxygen species: a common pathway for PAR1- and PAR2-mediated tissue factor induction in human endothelial cells. J Thromb Haemost 7, 206–216 (2009).
13. Suen, J. Y. et al. Profiling gene expression induced by protease-activated receptor 2 (PAR2) activation in human kidney cells. PLoS One 5, e13809 (2010).
14. Chen, W. et al. Conversion of Peripheral CD4+CD25− Naive T Cells to CD4+CD25+ Regulatory T Cells by TGF-beta Induction of Transcription Factor Foxp3. J Exp Med 198, 1875–1886 (2003).
15. Chen, X. et al. Intestinal epithelial cell-derived integrin alpha v beta 6 plays an important role in the induction of regulatory T cells and inhibits an antigen-specific Th2 response. J Leukoc Biol 90, 751–759 (2011).
16. Kurotaki, D. et al. CSF-1-Dependent Red Pulp Macrophages Regulate CD4 T Cell Responses. J Immunol 186, 2229–2237 (2011).
17. He, S. H. et al. Interferon-lambda Mediates Oral Tolerance and Inhibits Antigen-Specific T-Helper 2 Cell-Mediated Inflammation in Mouse Intestine. Gastroenterology 141, 249–258 (2011).
18. Yang, X. O. et al. Molecular Antagonism and Plasticity of Regulatory and Inflammatory T Cell Programs. Immunity 29, 44–56 (2008).
19. Esposito, M. et al. IL-17- and IFN-gamma-secreting Foxp3+ T cells infiltrate the target tissue in experimental autoimmunity. J Immunol 185, 7467–7473 (2010).
20. Beyer, M. & Schulze, J. L. Plasticity of Treg cells: Is reprogramming of Treg cells possible in the presence of FOXP3? Int Immunopharmacol 11, 555–560 (2011).
21. Chung, Y. et al. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. Nat Med 17, 983–988 (2011).
22. Harris, M. B., Mostecki, J. & Rothman, P. B. Repression of an Interleukin-4-responsive Promoter Requires Cooperative BCL-6 Function. J Biol Chem 280, 13114–13121 (2005).
23. Kusam, S., Toney, L. M., Sato, H. & Dent, A. L. Inhibition of Th2 Differentiation and GATA-3 Expression by BCL-6. J Immunol 170, 2435–2441 (2003).
24. Wan, Y. Y. & Flavell, R. A. The roles for cytokines in the generation and maintenance of regulatory T cells. Immunity 41, 114–130 (2006).
25. Hamilton, M. J. et al. Essential role for mast cell tryptase in acute experimental colitis. Proc Natl Acad Sci U S A 108, 290–295 (2011).
26. Shin, K. et al. 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 (2008).
27. Rodewald, H. R. & Feyerabend, T. Widespread Immunological Functions of Mast Cells: Fact or Fiction? Immunity 37, 13–24 (2012).

Acknowledgments
This study was supported by grants from the Natural Science Foundation of SZU (No. 008004), the innovation of science and Technology Commission of Shenzhen Municipality (JCYJ20120613172599904), the Natural Science Foundation of China (No. 30871752, 31070799 and 81373176) and the Key Laboratory Project of Shenzhen (No. SW201110010).

Author contributions
Z.Q.L., J.P.S., X.L., H.P.Z., X.C., Z.G.L. and P.Y.Z. were involved in performing the experiments, analysis of the data and reviewed the manuscript. P.C.Y. designed the project, supervised the experiments and wrote the manuscript.

Additional information
Supplementary information accompanies this paper at http://www.nature.com/scientificreports

How to cite this article: Liu, Z.-Q. et al. Mast cell-derived serine proteinase regulates T helper 2 polarization. Sci. Rep. 4, 4649; DOI:10.1038/srep04649 (2014).

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. The images in this article are included in the article’s Creative Commons license, unless indicated otherwise in the image credit; if the image is not included under the Creative Commons license, users will need to obtain permission from the license holder in order to reproduce the image. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/3.0/