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Mast cell-derived serine proteinase regulates T helper 2 polarization

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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 bedsides 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[1]. Mast cells play a critical role in the establishment of the organ or tissue transplantation tolerance[2,3]. Yet, whether mast cells play a role in regulation of the T helper (Th)2 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[4]. 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[5] and the B-cell lymphoma 6 protein (Bcl-6)[6]; the latter can be regulated in the processes of Th2 response[7]. Whether mast cell-derived serine protease modulates the expression of Bcl-6 in Th2 cells is unclear.

In line with previous studies[5,6], 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

Adoptive 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[2,3], which implies that mast cells may be able to regulate the abnormal immune responses. To test the hypothesis, we adoptively transferred OVA-specific $CD4^+$ $C25^-$ T cells (10^6 cells/mouse, from OTII mice; labelled with carboxyfluorescein succinimidyl ester; CFSE) to mast cell-deficient $Kit^{W\text{-}sh}/Kit^{W\text{-}sh}$ ($W^{\text{-}sh}$) 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 naïve 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-

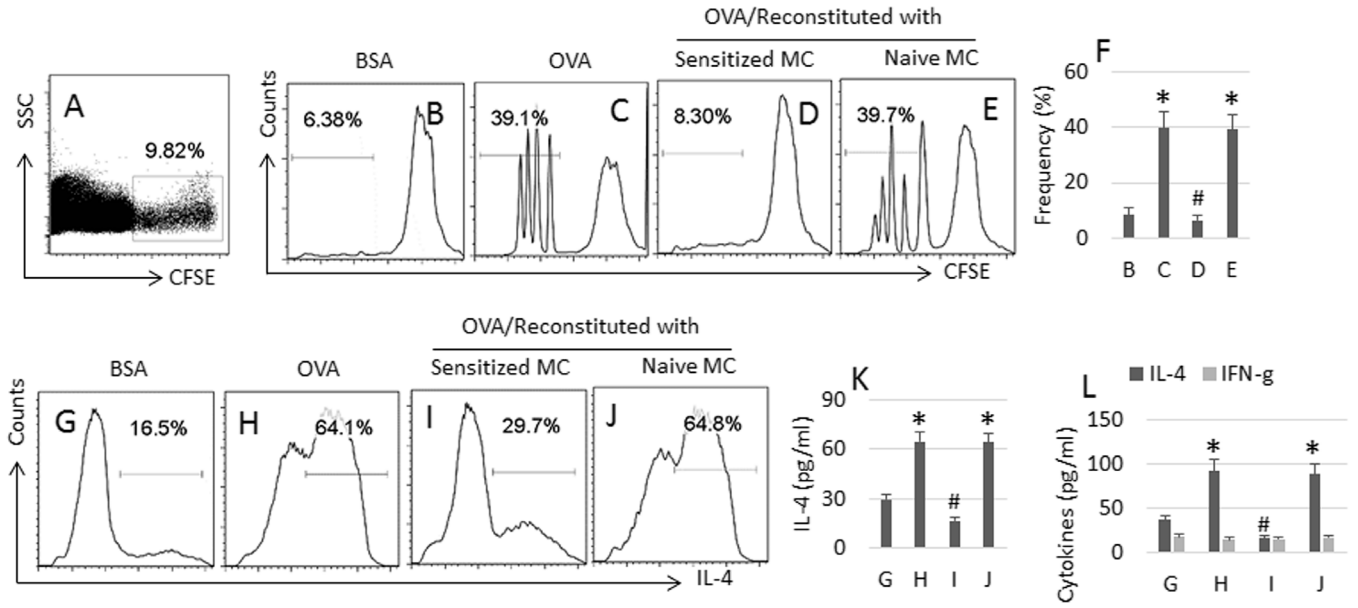

Figure 1 | Mast cells trigger the immune regulatory response. $CD4^+$ $CD25^-$ T cells (isolated from OTII mice) were labelled with CFSE, and adoptively transferred to mast cell-deficient W^{-sh} mice (5×10^6 cells/mouse). The mice were reconstituted with OVA-specific IgE-sensitized BMMCs, or naïve BMMC. 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^+ 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-γ in the culture supernatant (assessed by ELISA). *, $p < 0.01$, compared with group E (F) or J (K, L). *, $p < 0.01$, compared with group B (F), or group G (K, L). The data represent 3 separate experiments.

labeled cells were gated first (Fig. 1A). The gated cells were analyzed for the frequency of proliferating $CD4^+$ T cells (by the CFSE-dilution assay). The results showed that treatment with a non-specific antigen (BSA) did not induce the T cell proliferation (Fig. 1B, F) while using specific antigen, OVA, markedly increased the $CD4^+$ T cell proliferation (Fig. 1C, F), which did not occur in mice reconstituted with OVA-specific IgE-sensitized mast cells (Fig. 1D, F). The fact implicates that the sensitized mast cells suppress the antigen specific $CD4^+$ T cell proliferation. To strengthen the results, we reconstituted the W^{-sh} mice with naïve mast cells and adoptive transfer with OVA-specific $CD4^+$ T cells. The challenge with OVA induced marked $CD4^+$ T cell proliferation (Fig. 1E, F). In addition, we also observed that the levels of IL-4, but not IFN-γ, in the supernatant were changed in parallel with the changes of $CD4^+$ T cell proliferation (Fig. 1L); similar results were obtained in assessing the IL-4 mRNA expression in the sorting $CD4^+$ T cells (Fig. S3).

Activation of mast cells increases the expression of Bcl-6 in $CD4^+$ T cells. We next cultured the OVA-specific IgE-sensitized BMMCs 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 BMMCs 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).

T cells express PAR2[5]; mast cell-derived mMCP-6 can cleave the PAR2 to regulate the functions of target cells. We next tested if the mast cell-increased Bcl-6 expression in $CD4^+$ T cells was mediated by PAR2. The PAR2 gene was knocked down in the $CD4^+$ T cells by the RNA interference (Fig. S5); the PAR2-deficient $CD4^+$ T cells were exposed to mast cells with the same procedures above. Indeed, the increase in Bcl-6 in the $CD4^+$ T cells was abrogated, which was mimicked by using the active peptides of PAR2 (Fig. 2A–B). The data indicate that mast cell-derived mMCP-6 can up-regulate the expression of Bcl-6 in $CD4^+$ T cells. In separate experiments, we pretreated the Th2 cells with p38 MAPK antagonist or ERK1/2 antagonist, which abolished the increases in Bcl-6 expression induced by mast cells (Fig. S4; Table S1). The results indicate that the activation of MAPK pathway is involved in the PAR2-increased Bcl-6 expression in Th2 cells.

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. BMMCs (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 BMMCs, the activated BMMCs 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

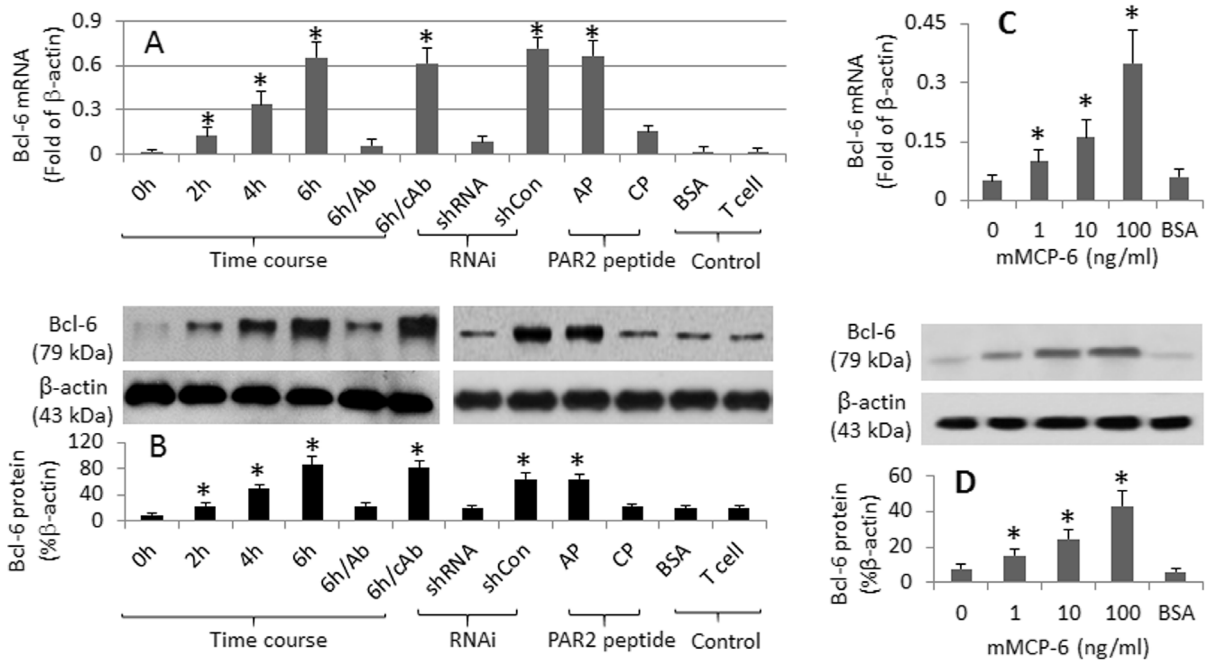

Figure 2 | Mast cells up-regulate the Bcl-6 expression in Th2 cells. IgE-sensitized BMMC were cultured with $CD4^+$ T cells (isolated from OTII mouse spleen; 10^6 cells/ml; BMMC : T cell = 1 : 10) in the presence of specific antigen OVA and DC (T cell : DC = 10 : 1) for 0–6 h with the following conditions: (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 (C) 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.

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) to the reconstituted W^{sh} 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 $CFSE^+$ $CD4^+$ T cells were gated first, the phenotypes were further analyzed. The results showed that in mast cell-null mice, about 4% transferred $CD4^+$ T cells were apoptotic (Fig. 4A, 4D), which was slightly increased ($p > 0.05$) in mice reconstituted with sensitized mast cells and challenged OVA (Fig. 4B, 4D), but markedly increased ($p < 0.01$) in mice reconstituted with naïve mast cells and challenged with OVA (Fig. 4C–D). On the other hand, we detected about 1.2% Tregs in the $CD4^+$ T cells isolated from the mast cell-null mice (Fig. 4E, 4H); the frequency of Tregs was increased markedly in the $CD4^+$ T cells isolated from mice reconstituted with sensitized mast cells (Fig. 4F, 4H), but not in those isolated from mice reconstituted with naïve mast cells (Fig. 4G–H).

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[8]. 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 $Foxp3^+$ Tregs. 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 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

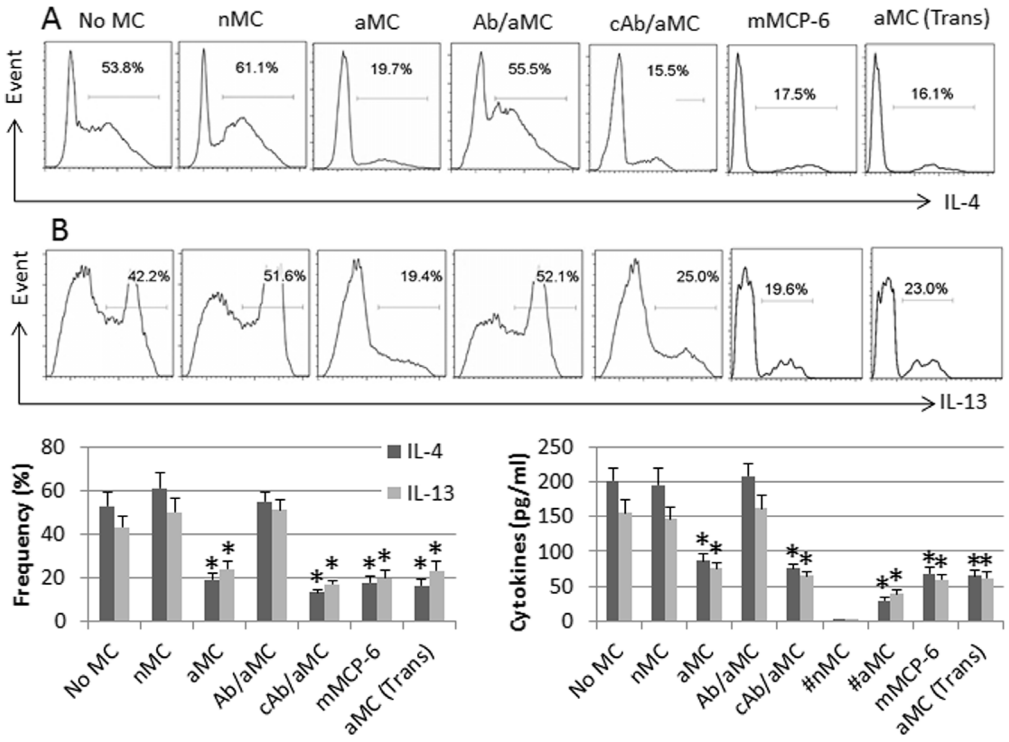

Figure 3 | **Mast cell activation suppresses Th2 responses.** The OTII $CD3^+$ $CD4^+$ $CD25^-$ cells were cultured with OVA-specific IgE sensitized BMMC in the presence of the specific antigen, OVA (to activate mast cells), and DC. A batch of the cells was pretreated with anti-mMCP-6 antibody [Ab; or treated with isotype IgG (cAb), using as a control]. The cells were collected on day 4, analyzed by flow cytometry by intracellular staining. (A and B), the histograms show the frequency of IL-4^+ (A) and IL-13^+ (B) T cells. (C), the bars show the summarized data in (A) and (B. D), the supernatants were analyzed by ELISA. The bars indicate the cytokine levels of IL-4 and IL-13 in the supernatants. The data in bar graphs were presented as mean ± SD from 3 experiments. *, $p < 0.01$, compared with nMC group. nMC indicates the not activated mast cells; aMC indicates the activated mast cells; the ''No T cell'' indicates no T cells in the culture.

(Fig. 6H). 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[9]. On the other hand, mast cells are also involved in the development of adaptive immunity[1]. The present data add novel information to mast cells' properties by showing that mast cells induce the intestinal Th2 cells to express

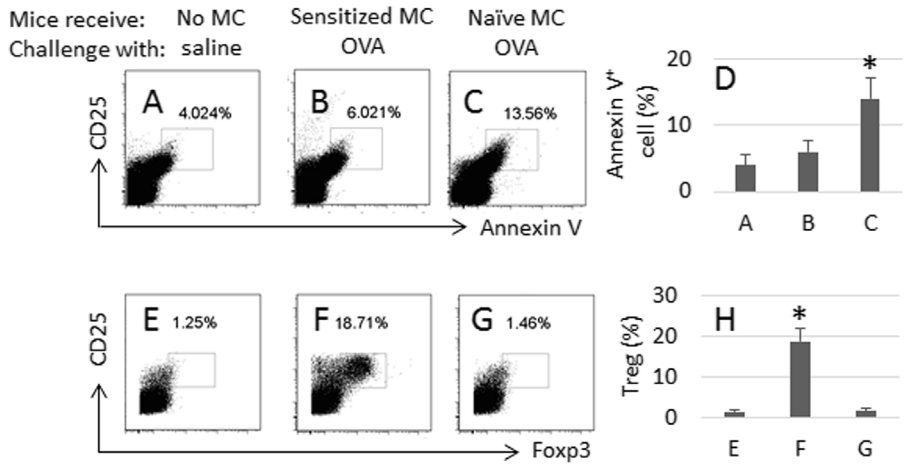

Figure 4 | **Activation of mast cells converts Th0 cells to Tregs.** The W^{sh} mice were reconstituted with OVA-specific IgE-sensitized BMMCs, or naïve BMMCs. $CD4^+$ $CD25^-$ T cells were isolated from the OTII mouse intestine, labelled with CFSE, and adoptively transferred to the mice (10^6 cells/mouse). Mice were fed with saline or OVA (5 mg/mouse) daily for one week. After sacrifice, LPMCs were prepared and stained with anti-Foxp3, anti-CD25 and annexin V (labelled with PE) reagent, and analyzed by flow cytometry. The $CFSE^+$ cells were gated first; the phenotypes of the $CFSE^+$ cells were further analyzed. The gated dot plots indicate the frequencies of apoptotic cells (A–C) or Tregs (E–G). The bar graphs (D, H) show the summarized data of dot plots. The data of bars are presented as mean ± SD. *, $p < 0.01$, compared with group A (D), or group E (H). Each group consisted of 6 mice. The data represent 6 separate experiments.

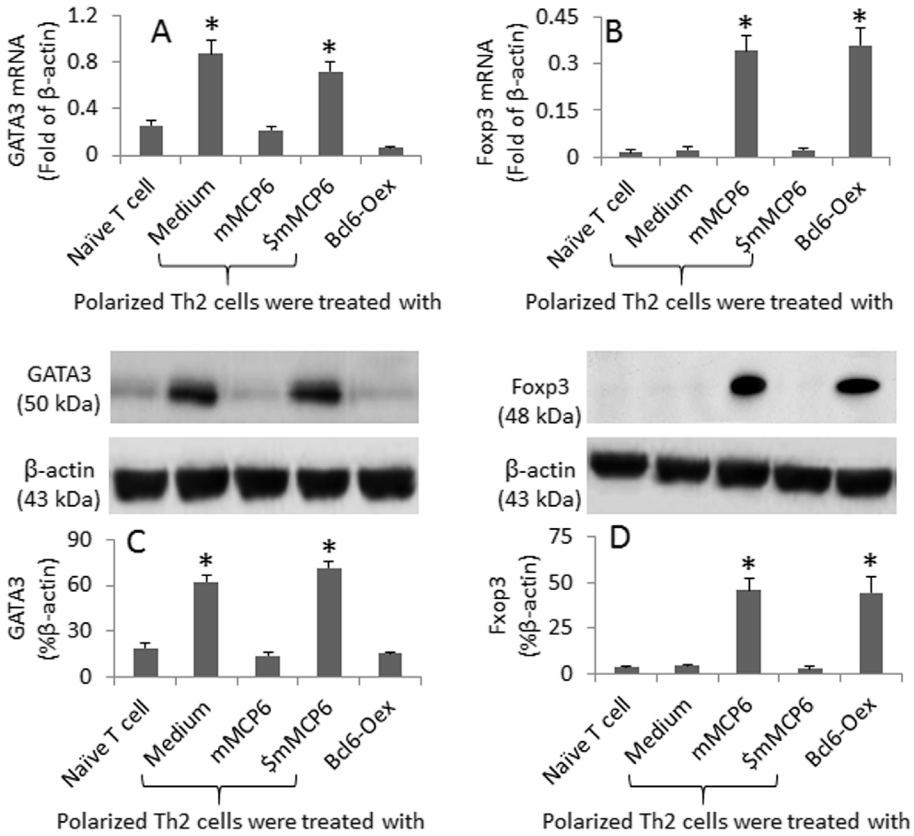

Figure 5 | mMCP-6 modulate the expression of GATA3 and Foxp3 in Th2 cells. From the OTII mouse spleen, we prepared naïve $CD4^+$ T cells and polarized Th2 cells. The polarized Th2 cells (10^6 cells/ml) were cultured in the presence of mMCP-6 (50 μM) for 48 h as denoted on the x axis. The cellular extracts 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 naive T cell group.

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[2,3].

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 of (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[10]; 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[11]; the activation of PAR2 increases intracellular reactive oxygen species also via the MAPK pathway[12]. 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[13]. 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.

The inducible Tregs can be induced from Th0 cells in a given cytokine environment. It is well known that the exposure to TGF-β can drive Th0 cells to differentiate into $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs[14]. The sources of TGF-β can be the antigen presenting cells such as dendritic cells[15] or macrophages[16]. Our recent work indicates the dendritic cell-derived IFN-λ can modulate the generation of Tregs in the intestine[17]. The present data provide a novel pathway in the induction of Tregs in the intestine by showing that mast cell activation can trigger the development of Tregs. It is noteworthy that the precursors of Tregs in the present study are not Th0 cells but Th2 cells. Although this is a novel finding that Th2 cells can be converted

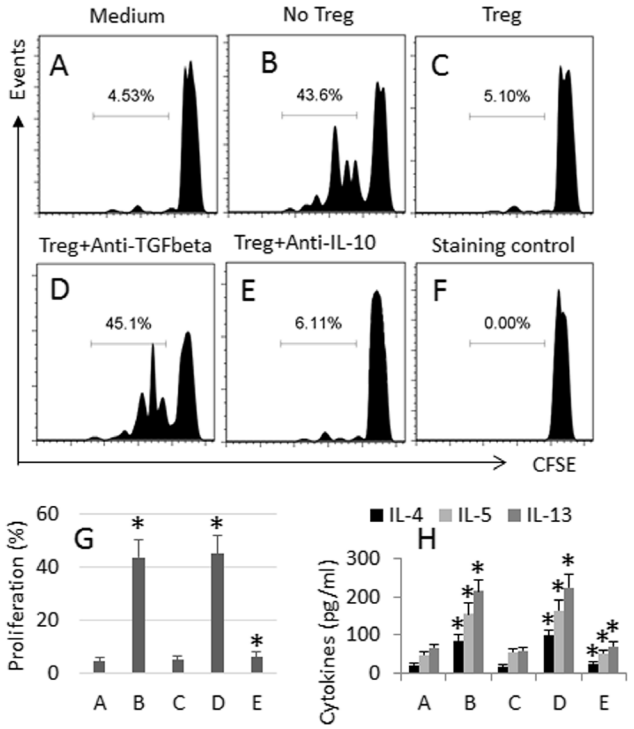

Figure 6 | The generated Tregs have an immune suppressor function. The generated Tregs, DCs and polarized OVA-specific Th2 cells (labelled with CFSE) were prepared as described in the text and cultured in the presence of OVA in culture for 3 days under the following conditions: (A) Medium, Th2 cells were cultured with medium. (B) No Treg, Th2 cells were cultured in the presence of OVA, but no Treg. (C) With Treg, Th2 cells were cultured in the presence of OVA and Treg. (D) Treg + aTGF-β, Th2 cells were cultured in the presence of OVA, Treg and anti-TGF-β pAb (200 ng/ml). (E) Treg + isoIgG, Th2 cells were cultured in the presence of OVA, Treg and isotype IgG (100 ng/ml, using as a control antibody). (A–E), the histograms indicate the frequency of Th2 cell proliferation. (F) is a CFSE-staining control. (G), the bars indicate the summarized data of (A–E). (H), the bars indicate the levels of IL-4, IL-5 and IL-13 in the culture supernatants. The labels of G and H are the same as that in the flow cytometry histograms. Data of (G) and (H) are presented as mean ± SD. *, $p < 0.01$, compared with group A. #, $p < 0.05$, compared with group B. The data represent 3 experiments.

to Tregs, the data are supported by several other reports that T helper effector cells can be converted to Foxp3$^+$ T cells and vice versa[18–20]. Our findings further emphasize the plasticity of T helper cells. In line with a recent report[21], the key molecule in the Treg-conversion in the present study is Bcl-6, a transcriptional repressor, that has been implicated to suppress the expression of IL-4 and the transcription factor GATA3 in other studies[22,23].

The immune responses, such as Th2 response, are originally protective activities 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 fulfil 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[14], IL-10[24], epithelial cell-derived αvβ6[15], 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 upregulated in Th2 cells by exposing to the activated-mast cells. Under physiological conditions, the GATA3 binds to Foxp3 promoter to prevent its transcription[8], 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[23]. 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[25]. Shin et al observed that mast cell-derived mMCP-6 was a critical molecule in recruiting eosinophils to expel parasites from the infected sites[26]. 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[27]. 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.

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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

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