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Mast Cells Down-Regulate CD4⁺CD25⁺ T Regulatory Cell Suppressor Function via Histamine H1 Receptor Interaction

Nicholas A. Forward,* Suzanne J. Furlong, * Yongjun Yang, ‡ Tong-Jun Lin, *‡ and David W. Hoskin2,*§

Mast cells promote both innate and acquired immune responses, but little is known about the effect of mast cells on T regulatory (Treg) cell function. In this study, we show for the first time that the capacity of murine CD4⁺CD25⁺ Treg cells to suppress in vitro proliferation by CD4⁺CD25⁻ T responder (Tresp) cells in response to anti-CD3/anti-CD28 mAb-coated beads was reduced in the presence of syngeneic bone marrow-derived mast cells (BMMC) activated by FcεR cross-linking. Activated BMMC culture supernatants or exogenous histamine also inhibited Treg cell suppressor function while the histamine H1 receptor-specific antagonist loratadine, restored Treg cell suppressor function in the presence of activated BMMC or activated BMMC culture supernatants. Moreover, treatment of Treg cells with loratadine, but not famotidine, rescued Treg cell suppressor function in the presence of exogenous histamine. In addition, the H1 receptor-specific agonist 2-pyridylethylamine dihydrochloride inhibited Treg cell suppressor function to an extent that was comparable to histamine, whereas the H2 receptor-specific antagonist amthamine dihydrobromide was without effect. Both Treg cells and Tresp cells expressed H1 receptors. Exposure to histamine caused Treg cells to express lower levels of CD25 and the Treg cell-specific transcription factor Foxp3. Taken together, these data indicate that BMMC-elaborated histamine inhibited Treg cell suppressor function by signaling through the H1 receptor. We suggest that histamine released as a result of mast cell activation by microbial products might cause a transient decrease in Treg cell suppressor function, thereby enhancing the development of protective immunity.


In recent years, the importance of several different T regulatory (Treg) cells subsets in the establishment and maintenance of immune tolerance have become increasingly apparent. Perhaps the best studied of these are “natural” Treg cells that develop in the thymus and are important endogenous regulators of immune responses to self- and foreign Ags (1, 2). The vast majority of naturally occurring CD4⁺ Treg cells constitutively express CD25 (IL-2Rα-chain) (3) and the Foxp3 transcription factor, which plays a critical role in Treg cell development and effector function (4–6). Endogenous Treg cells can potentially suppress the in vitro activation of other T cell subsets through contact-dependent processes that have been suggested to involve CTLA-4 (7), membrane-bound TGF-β (8), and/or the release of granzyme B, without or with a requirement for perforin (9, 10). Treg cells also express the ectonucleotidases CD39 and CD73, which allows Treg cells to generate adenosine and thereby mediate immune suppression (11). Although Treg cell suppressor function appears to be strictly contact-dependent in vitro, immunosuppressive cytokines such as IL-10 and TGF-β contribute to Treg suppressor activity in vivo (12). The ability of Treg cells to prevent the activation of autoreactive T cells and limit nonspecific bystander damage by modulating immune responses to foreign Ags is of obvious benefit; nevertheless, the initiation of protective immune responses toward pathogens must involve processes that overcome or at least diminish the immunosuppressive activity of endogenous Treg cells.

Although mast cells are traditionally viewed in the context of allergy and asthma, the ability of mast cells to become activated by diverse stimuli and to produce a wide variety of mediators makes them important players in many other physiological processes (13). Mast cells are an important component of the innate immune system, acting as sentinel cells that detect infecting microorganisms via pattern recognition molecules such as TLR (14). The wide variety of cytokines, chemokines, and other mediators, including histamine, that are produced and released by mast cells following their activation are believed to promote the recruitment of other immune effector cells and modulate their activity. Since mast cells are first-line responders to microbial infections and are present in environments that may also contain endogenous Treg cells, it seems likely that mast cells and Treg cells might affect each others’ function. However, only limited information is available on the interactions that take place between mast cells and Treg cells. In a mouse model of sepsis, adoptive transfer of Treg cells correlates with an increase in mast cell numbers in the peritoneum (15), whereas mast cell recruitment to skin allografts in response to IL-9 produced by Treg cells is essential for the establishment of tolerance to alloanergens (16). In addition, a recent study (17) showed that Treg cells down-regulate FcεRI expression by mast cells in vitro.
through a contact-dependent mechanism and also down-regulate IgE-mediated leukotriene C4 production by mast cells. Although these studies indicate that Treg cells possess the capacity to recruit mast cells and regulate their activation, the effect(s) that mast cells have on Treg cell suppressor function has not yet been investigated.

In this study, we show for the first time that histamine released by activated murine bone marrow-derived mast cells (BMMC) inhibited the suppressor function of CD4+CD25+ Treg cells by signaling through the H1 receptor. In addition, the histamine-induced decrease in Treg cell suppressor function was associated with reduced expression of CD25 and the Treg cell-specific transcription factor Foxp3. Activation-induced release of histamine by mast cells may promote the development of protective immune responses to infecting microorganisms by transiently down-regulating endogenous Treg cell activity.

Materials and Methods

Mice

For all experiments, 6- to 8-wk-old female C57BL/6 mice (Charles River Laboratories Canada) were used. Animals were cared for and housed in the Carleton Animal Care Facility (Dalhousie University) in accordance with Canadian Council on Animal Care guidelines.

Isolation and culture of T cells

CD4+CD25+ Treg cells were isolated from mouse spleen cell preparations by negative selection for CD4+ cells and positive selection for CD25+ cells using a MACS mouse Treg cell isolation kit (Miltenyi Biotec). The CD4+CD25+ T cell population was retained for use as T responder (Tresp) cells in suppression assays. The purity of the MACS-isolated T cell fraction was confirmed by two-color flow cytometric analysis using anti-CD25-PE mAb (Miltenyi Biotec) and anti-CD4-FTC mAb (eBioscience). Purity of the CD4+CD25+ Treg cell fraction and CD4+CD25+ Tresp cell fraction was typically 90 and 95%, respectively. For all experiments, T cells were cultured at 37°C/5% CO2/95% humidity in RPMI 1640 medium (Sigma-Aldrich Canada) supplemented with 5% heat-inactivated FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM l-glutamine, and 5 mM HEPES (all from Invitrogen).

BMMC differentiation and IgE priming

BMMC were obtained by culturing bone marrow cells from murine femurs and tibias in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 5% heat-inactivated FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM l-glutamine, and 5 mM HEPES (all from Invitrogen). For all experiments, 6- to 8-wk-old female C57BL/6 mice (Charles River Laboratories Canada) were used. Animals were cared for and housed in the Carleton Animal Care Facility (Dalhousie University) in accordance with Canadian Council on Animal Care guidelines.

T cell proliferation assays

CD4+CD25+ Tresp cells (1 × 10⁶) and/or CD4+CD25+ Treg cells (2 × 10⁶) were cultured in quadruplicate wells of a 96-well microtiter plate with either anti-CD3/anti-CD28 mAb-coated T cell expander beads (5 × 10⁵), and/or anti-TNP IgE-primed BMMC (2 × 10⁵) and 10 ng/ml TNP-BSA for 24 h. Culture supernatants were then harvested, and IL-2 levels in quadruplicate samples were determined using an IL-2 OptEIA ELISA kit (BD Biosciences).

Flow cytometry

CD4+CD25+ Treg cells were stained for 30 min at 4°C with 5 µg/ml anti-CD25-FITC mAb (Cedarlane Laboratories) or fixed, permeabilized, and stained with 1 µg/ml anti-Foxp3-FTC mAb (eBioscience), according to the manufacturers’ specifications. Control cells were stained with the appropriate FITC-conjugated isotype control Ab. Cells were analyzed using a FACSCalibur flow cytometer and CellQuest software.

Real-time quantitative RT-PCR

Total RNA was extracted from equal numbers of Tresp cells and Treg cells using TRIzol reagent and reverse transcribed using Superscript II RNase H reverse transcriptase (both from Invitrogen), according to the manufacturer’s instructions. Histamine H1 receptor mRNA was quantified by TaqMan MGB Probe and TaqMan Master Mix (both from Applied Biosystems) on the ABI Prism 7000 sequence detection system (95°C for 10 min, followed by 40 cycles of 95°C for 5 s and 60°C for 1 min). GAPDH mRNA was analyzed using the TaqMan Rodent GAPDH Control Reagents (Applied Biosystems). Data were analyzed using the relative standard curve method, according to the manufacturer’s protocol. The result was expressed as a ratio of histamine H1 receptor to GAPDH mRNA. In addition, PCR products were resolved on 2% agarose gel and visualized under UV light after staining with etidium bromide. Western blotting

T cells (2.5 × 10⁵) were pelleted and lysed with ice-cold lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 50 mM NaHPO₄, 0.25% sodium deoxycholate, 0.1% Nonidet P-40, 5 mM EDTA, and 5 mM EGTA) containing freshly added protease and phosphatase inhibitors (5 µg/ml leupeptin, 5 µg/ml pepstatin, 10 µM aprotinin, 1 mM PMSF, 10 mM NaF, 1 mM DTT, and 100 µM NaVO₄). Protein was quantified using Bio-Rad Protein Assay reagent (Bio-Rad) and diluted with SDS-PAGE sample buffer (200 mM Tris-HCl (pH 6.8), 30% glycerol, 6% SDS, 15% 2-ME, and 0.01% bromophenol blue). Samples were then boiled for 5 min and stored at −80°C. Protein lysates were thawed on ice; proteins were resolved by SDS-PAGE (20 µg protein/lane) and electrotransferred onto nitrocellulose membranes. Membranes were blocked in TBST (20 mM Tris-HCl (pH 7.6), 200 mM NaCl, and 0.05% Tween 20) containing 5% fat-free milk overnight at 4°C, then washed with TBST and incubated with anti-histamine H1 receptor mAb (clone H-300; Santa Cruz Biotechnology) diluted 1/200 in blocking solution overnight at 4°C. Membranes were washed with fresh TBST and incubated with HRP-conjugated goat anti-rabbit Ab (Santa Cruz Biotechnology) diluted 1/1000 in blocking solution for 1 h at room temperature. Membranes were washed with TBST, reacted with ECL reagents (GE Healthcare) for 1 min, and exposed to x-ray film.

To confirm equal protein loading, membranes were incubated in stripping buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, and 100 mM 2-ME at 37°C for 30 min to remove Abs, then washed with fresh TBST and sequentially probed with anti-actin mAb (clone I-19; Santa Cruz Biotechnology) and anti-actin rabbit Ab (Santa Cruz Biotechnology) diluted 1/1000. Statistical analysis

Data were analyzed using the Instat statistics program (GraphPad Software). Statistical comparisons were performed using Student’s t test or ANOVA with the Tukey-Kramer multiple comparisons posttest.

Results

BMMC inhibit CD4+CD25+ Treg cell suppressor function

To determine the capacity of mast cells to modulate the function of CD4+CD25+ Treg cells, CD4+CD25+ Tresp cells were stimulated with anti-CD3/anti-CD28 mAb-coated beads alone or in combination with CD4+CD25+ Treg cells (5:1 ratio) and/or anti-TNP IgE-primed BMMC and TNP-BSA. As expected, Treg cells inhibited Tresp cell proliferation (p < 0.001), as determined by [³H]Tdr...
incorporation (Fig. 1A). TNP-activated BMMC did not affect $T_{\text{resp}}$ cell proliferation. Importantly, there was a marked and significant ($p < 0.001$) reduction in $T_{\text{reg}}$ cell-mediated suppression of $T_{\text{resp}}$ cell proliferation in the presence of TNP-activated BMMC. Neither $T_{\text{resp}}$ cell proliferation nor $T_{\text{reg}}$ cell-mediated suppression of $T_{\text{resp}}$ cell proliferation was altered in the presence of unactivated BMMC (data not shown). Because as many as three different cell populations were present in our assay system, it was important to confirm that changes in $[^{3}H]TdR$ incorporation were due to increased $T_{\text{resp}}$ cell proliferation and were not caused by increased proliferation of $T_{\text{reg}}$ cells and/or BMMC. $T_{\text{reg}}$ cells were therefore labeled with Oregon Green 488 dye, which allowed us to specifically measure $T_{\text{reg}}$ cell division by flow cytometry. As shown in Fig. 1B, anti-CD3/anti-CD28 mAb-coated bead-stimulated $T_{\text{resp}}$ cells that were cocultured with $T_{\text{reg}}$ cells showed a smaller percentage of proliferating $T_{\text{resp}}$ cells and fewer rounds of cell division over a 72-h period in comparison to $T_{\text{resp}}$ cells activated in the absence of $T_{\text{reg}}$ cells. Furthermore, the addition of anti-TNP IgE-primed BMMC and TNP-BSA to $T_{\text{reg}}$ cell-T$_{\text{resp}}$ cell cocultures restored $T_{\text{resp}}$ cell proliferation to levels that were equivalent to that seen when $T_{\text{resp}}$ cells were activated in the absence of $T_{\text{reg}}$ cells. TNP-activated BMMC had no effect on $T_{\text{resp}}$ cell division in the absence of $T_{\text{reg}}$ cells. Unactivated BMMC did not affect $T_{\text{resp}}$ cell division in the absence or presence of $T_{\text{reg}}$ cells (data not shown). Taken together, these data show that activated BMMC down-regulated the suppressor function of CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cells.

Supernatant from activated BMMC cultures abrogates $T_{\text{reg}}$ cell suppressor function

To determine whether the inhibitory effect of mast cells on CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cell function was due to a soluble factor, we next examined the effect of activated BMMC culture supernatant on CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cell function. Cell-free culture supernatants were harvested after exposing anti-TNP IgE-primed BMMC to TNP-BSA for 24 h. CD4$^{+}$CD25$^{+}$ $T_{\text{resp}}$ cells were then stimulated with anti-CD3/anti-CD28 mAb-coated beads either alone or in combination with CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cells (5:1 ratio) in the absence or presence of supernatant from cultures of activated BMMC. As shown in Fig. 2A, $T_{\text{reg}}$ cell suppressor function in a $[^{3}H]TdR$ incorporation assay was abrogated in the presence of activated BMMC culture supernatant ($p < 0.001$). BMMC culture supernatant by itself did not affect $T_{\text{resp}}$ cell proliferation. Similarly, the addition of activated BMMC culture supernatant to cocultures of Oregon Green 488 dye-labeled $T_{\text{resp}}$ cells and unlabeled $T_{\text{reg}}$ cells resulted in reduced $T_{\text{reg}}$ cell-mediated suppression of $T_{\text{resp}}$ cell division (Fig. 2B). Collectively, these data demonstrate that the inhibitory effect of BMMC on $T_{\text{reg}}$ cell function was mediated by a BMMC-derived soluble factor.

Histamine inhibits CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cell suppressor function

FcεR cross-linking causes mast cells to release large amounts of histamine, which is a soluble factor with numerous effects on the immune system (19). To determine whether histamine was able to inhibit CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cell suppressor function, CD4$^{+}$CD25$^{+}$ $T_{\text{resp}}$ cells were stimulated with anti-CD3/anti-CD28 mAb-coated beads in the presence of increasing doses of histamine (0–10 μM), either alone or in coculture with CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cells (5:1 ratio). As shown in Fig. 3A, $T_{\text{reg}}$ cell-mediated suppression of $T_{\text{resp}}$ cell proliferation decreased with increasing doses of histamine, whereas histamine had no effect on $T_{\text{resp}}$ cell proliferation in the absence of $T_{\text{reg}}$ cells. Histamine (10 μM) did not affect the proliferation or viability of $T_{\text{reg}}$ cells (data not shown). Since histamine could interfere with $T_{\text{reg}}$ cell suppressor function either by

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**FIGURE 1.** Inhibition of CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cell suppressor function by activated BMMC. A, CD4$^{+}$CD25$^{+}$ $T_{\text{resp}}$ cells ($1 	imes 10^5$) were stimulated with anti-CD3/anti-CD28 mAb-coated beads ($5 	imes 10^6$) alone or in combination with CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cells ($2 	imes 10^5$). Where indicated, anti-TNP-IgE-primed BMMC ($2 	imes 10^5$) and TNP-BSA (10 ng/ml) were also added to the cultures. After 48 h of incubation, cultures were pulsed with $[^{3}H]TdR$ for the last 6 h of culture. Data from an experiment that is representative of three independent experiments are shown as mean cpm ± SD of quadruplicate cultures. B, Oregon Green 488-labeled CD4$^{+}$CD25$^{+}$ $T_{\text{resp}}$ cells ($1 	imes 10^5$) were stimulated with anti-CD3/anti-CD28 mAb-coated beads ($5 	imes 10^6$) alone or in combination with unlabeled CD4$^{+}$CD25$^{+}$ $T_{\text{reg}}$ cells ($2 	imes 10^5$). Where indicated, anti-TNP IgE-primed BMMC ($2 	imes 10^5$) and TNP-BSA (10 ng/ml) were also added to the cultures. After 72 h of incubation, $T_{\text{resp}}$ cell proliferation was determined by flow cytometry. Data from a representative experiment. Cumulative data from three independent experiments are shown as percent dividing $T_{\text{resp}}$ cells ± SEM. Statistical significance was determined by ANOVA with the Tukey-Kramer multiple comparisons posttest.
directly inhibiting the T<sub>reg</sub> cells or by rendering T<sub>resp</sub> cells refractory to T<sub>reg</sub> cell-mediated suppression, we pretreated T<sub>resp</sub> cells or T<sub>reg</sub> cells with histamine (10 μM) for 30 min and then washed extensively before assaying cell proliferation by [H]<sup>3</sup>TdR incorporation. T<sub>resp</sub> cells that were pretreated with histamine proliferated normally following stimulation with anti-CD3/anti-CD28 mAb-coated beads and were suppressed by untreated CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells (Fig. 3B). In contrast, T<sub>reg</sub> cells that were pretreated with histamine failed to inhibit the proliferation of untreated or histamine-pretreated T<sub>resp</sub> cells. These data indicate that histamine directly inhibited T<sub>reg</sub> cell suppressor function rather than rendering T<sub>resp</sub> cells refractory to T<sub>reg</sub> cell-mediated suppression. We also investigated the effect of histamine on T<sub>reg</sub> cell-mediated inhibition of IL-2 production by anti-CD3/anti-CD28 mAb-coated bead-stimulated T<sub>resp</sub> cells. Although histamine had no effect on IL-2 production by T<sub>resp</sub> cells in the absence of T<sub>reg</sub> cells, T<sub>reg</sub> cell-mediated suppression of IL-2 production by T<sub>resp</sub> cells was abrogated in the presence of histamine (p < 0.001; Fig. 3C).

**H<sub>1</sub> receptor antagonism prevents BMMC-mediated inhibition of T<sub>reg</sub> cell suppressor function**

To determine whether histamine was responsible for BMMC-mediated inhibition of CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cell suppressor function, we treated cocultures of CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells, CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells, and anti-TNP IgE-primed BMMC with the histamine H<sub>1</sub> receptor antagonist loratadine (20) or the histamine H<sub>2</sub> receptor antagonist famotidine (21) before the addition of TNP-BSA and anti-CD3/anti-CD28 mAb-coated beads. Fig. 4A shows significant (p < 0.001) rescue of T<sub>reg</sub> suppressor function in the presence of activated BMMC when loratadine was also present; however, there was no rescue by famotidine. Neither loratadine nor famotidine affected T<sub>resp</sub> cell proliferation in the absence of T<sub>reg</sub> cells. Similarly, treatment with loratadine, but not famotidine, significantly (p < 0.001) reduced the inhibitory effect of activated BMMC culture supernatant on T<sub>reg</sub> cell suppressor function (Fig. 4B). Taken together, these data suggest that histamine was responsible for the inhibitory effect of activated BMMC on T<sub>reg</sub> cell suppressor function and that BMMC-derived histamine acts on T<sub>reg</sub> cells via the H<sub>1</sub> receptor.

To confirm that histamine inhibited T<sub>reg</sub> cell function by acting through H<sub>1</sub> receptors, we showed that exposure to increasing doses (2.5–10 μM) of loratadine resulted in a dose-dependent rescue of T<sub>reg</sub> cell suppressor function in the presence of histamine (Fig. 5A). In contrast, the highest dose of famotidine (10 μM) had only a slight effect on T<sub>reg</sub> cell suppressor function in the presence of histamine. We also examined the effects of 2-PEA, a highly selective H<sub>1</sub> receptor agonist (22), and ADHB, a highly selective H<sub>2</sub> receptor agonist (23), on T<sub>reg</sub> cell-mediated inhibition of T<sub>resp</sub> cell proliferation in response to anti-CD3/anti-CD28 mAb-coated bead stimulation. Exposure to increasing doses of 2-PEA to T<sub>reg</sub> cell-T<sub>resp</sub> cell cocultures resulted in a dose-dependent decrease in T<sub>reg</sub> cell-mediated suppression, whereas ADHB, even at the highest dose used, had little effect on T<sub>reg</sub> cell function (Fig. 5B). Collectively, these data indicate that the inhibitory effect of histamine on T<sub>reg</sub> cell suppressor function was mediated exclusively through H<sub>1</sub> receptors.

**H<sub>1</sub> receptor expression by T<sub>reg</sub> cells and T<sub>resp</sub> cells**

Although mouse T cells are known to express H<sub>1</sub> receptors (24), H<sub>1</sub> receptor expression by CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells has not yet been examined and compared with CD4<sup>+</sup>CD25<sup>+</sup> T<sub>resp</sub> cells. Real-time quantitative RT-PCR showed that H<sub>1</sub> receptor mRNA expression by T<sub>reg</sub> cells exceeded that of T<sub>reg</sub> cells by ~2-fold.
However, similar levels of H1 receptor protein were expressed by T resp cells and T reg cells (p > 0.05; Fig. 6B).

Histamine down-regulates CD25 and Foxp3 expression by Treg cells

CD4+CD25+ Treg cells are characterized by the constitutive expression of the high-affinity IL-2R α-chain, CD25 (3). Moreover,
IL-2 is important for the development, maintenance, and activation of Treg cells (25–27). We therefore examined the effect of histamine on CD25 expression by Treg cells. After 24 h of culture in the absence or presence of 10 μM histamine, Treg cells were stained for CD25 and analyzed by flow cytometry. In comparison to untreated Treg cells, histamine-treated Treg cells showed a marked decrease in CD25 expression (mean fluorescence intensity (MFI) 105 vs 27 ± 13, p < 0.01; Fig. 7A). We also determined the effect of histamine on Treg cell expression of the forkhead box/winged helix transcription factor Foxp3, which is important for Treg cell development and programming Treg cell suppressor function (4–6). Fig. 7B shows that 24 h of exposure to 10 μM histamine caused a marked decrease in Foxp3 expression in comparison to untreated Treg cells (MFI 20 ± 9 vs 62 ± 8, p < 0.01). Treg cell expression of CD25 and Foxp3 was therefore down-regulated by histamine. The inhibitory effect of histamine on CD25 and Foxp3 expression by Treg cells was not evident at earlier time points (2 or 10 h), nor was it transient since washing out histamine from Treg cell cultures after 30 min of exposure did not prevent CD25 and Foxp3 down-regulation at the 24-h time point (data not shown).

**Discussion**

There has been a paucity of information to date regarding the effect that mast cells have on Treg cell function. We show here, for the first time, that histamine released by FcεR-activated BMMC abrogated the suppressor function of CD4+CD25+ Treg cells. Activated BMMC potently inhibited the suppressor function of CD4+CD25+ Treg cells through a mechanism that involved H1 histamine receptor signaling, because the H1 receptor antagonist loratadine blocked the inhibitory effect of activated BMMC,
activated BMMC culture supernatant, or histamine on T_{reg} cell function. Furthermore, the H1 receptor agonist 2-PEA mimicked histamine-mediated inhibition of T_{reg} cell function. In contrast, H2 receptor antagonism with famotidine did not substantially block activated BMMC, activated BMMC culture supernatant, or histamine-mediated inhibition of T_{reg} cell function, and the H2 receptor agonist ADHB had little effect on T_{reg} cell function. Although both CD4^{+}CD25^{+} T_{reg} cells and CD4^{+}CD25^{-} T_{resp} cells expressed H1 receptors, histamine acted on T_{reg} cells rather than on T_{resp} cells to render them refractory to T_{reg} cell-mediated suppression because the proliferation of T_{resp} cells that were pretreated with histamine and then washed was potently suppressed by untreated T_{reg} cells, whereas T_{reg} cells that were pretreated with histamine and then washed did not inhibit the proliferation of untreated T_{resp} cells.

T_{reg} cells are dependent on IL-2 production by nonregulatory T cells because T_{reg} cell-expressed Foxp3 interacts with and prevents NFAT from binding to the IL-2 promoter (28). Indeed, IL-2 is critical for the activation and function of CD4^{+}CD25^{+} T_{reg} cells (12, 26). It has been suggested that constitutive high-level expression of CD25 may allow T_{reg} cells to inhibit the proliferation of T_{resp} cells by outcompeting T_{resp} cells for available IL-2 (27). Decreased CD25 expression by histamine-treated T_{reg} cells might therefore impact negatively on their ability to sequester T_{resp} cell-secreted IL-2. In line with this, IL-2 in culture supernatant was markedly decreased when T_{reg} cells were activated in the presence of T_{reg} cells but was restored to control levels following the addition of histamine to T_{resp} cell-T_{reg} cell cocultures. However, alternative interpretations for reduced IL-2 levels in the presence of CD4^{+}CD25^{+} T_{reg} cells must be considered since CD4^{+}CD25^{+} T_{reg} cells also suppress IL-2 mRNA synthesis by CD4^{+}CD25^{-} T_{resp} cells (29), as well as interfering with IL-2R signal transduction (30). In this regard, it is noteworthy that the suppressor function of T_{reg} cells is programmed by the Foxp3 transcription factor (5, 6), which also controls constitutive expression of CD25 by T_{reg} cells (6, 31, 32). Moreover, Foxp3 levels correlate with the suppressor capacity of CD4^{+}CD25^{+} T_{reg} cells (33). The virtual ablation of Foxp3 expression by T_{reg} cells in the presence of histamine might therefore account for the BMMC-mediated reduction in T_{reg} cell suppressor function. Interestingly, IL-2 is important for the maintenance of Foxp3 expression by T_{reg} cells (34). It follows then that reduced CD25 expression by T_{reg} cells in the presence of histamine may compromise their ability to use IL-2, thereby depriving T_{reg} cells of signals needed for the maintenance of Foxp3 expression. However, it is important to note that CD25-deficient T_{reg} cells that express IL-2R β- and γ-chains remain responsive to IL-2 (35). Moreover, IL-7 and IL-15 are sufficient to maintain Foxp3 expression by T_{reg} cells in the absence of IL-2 (36). Because CD25 expression is not always necessary for the induction of T_{reg} cell suppressor function, it is possible that histamine inhibited CD4^{+}CD25^{+} T_{reg} cell function by direct suppression of Foxp3 expression rather than by down-regulating constitutive T_{reg} cell expression of CD25 with an attendant decrease in Foxp3. Interestingly, the inhibitory effect of histamine on CD25 and Foxp3 expression by CD4^{+}CD25^{+} T_{reg} cells was not reversed when histamine was removed, which was consistent with the loss of suppressor function by T_{reg} cells that were pretreated with histamine and then washed before addition to culture.

A number of mast cell-derived mediators are known to regulate the activity of effector T cells. For instance, mast cell-produced TNF-α up-regulates vascular cell adhesion molecule expression by mouse endothelial cells, thereby promoting T cell accumulation in the inflamed footpads of mice treated with the mast cell-activating compound 48/80 (37). In addition, the proliferation of murine CD4^{+} T cells that are suboptimally activated with anti-CD3 mAb in the absence of CD28 costimulation is enhanced in the presence of FcɛRI-activated BMMC (38). On the basis of our findings, it is conceivable that BMMC-mediated inhibition of T_{reg} cell suppressor function contributed to enhanced CD4^{+} T cell proliferation in the absence of costimulation because the authors did not remove...
CD4\(^+\)CD25\(^+\) \(T_{reg}\) cells from their responder population. Histamine signaling through the H1 receptor is reported to enhance proliferative responses and IFN-\(\gamma\) production by CD4\(^+\) Th1 cells (39); however, increased CD4\(^+\) T cell proliferation and cytokine production may have been caused by histamine H1 receptor-mediated inhibition of endogenous CD4\(^+\)CD25\(^+\) \(T_{reg}\) cells contained within the CD4\(^+\) responder T cell population. These observations underscore the need to consider the possible effect(s) of endogenous CD4\(^+\)CD25\(^+\) \(T_{reg}\) cells when using CD4\(^+\) T cells that are heterogeneous in terms of their CD25 expression as a readout system.

Although \(T_{reg}\) cells constitute an important mechanism for regulating immune responses to both self- and foreign Ags (1, 2), optimal development of protective immune responses against invading microorganisms requires transient modulation of \(T_{reg}\) cell activity. For example, TLR2 and TLR8 ligands directly inhibit \(T_{reg}\) cell suppressor activity (40, 41), whereas signaling via TLR9 renders rat CD4\(^+\)CD25\(^+\) \(T_{reg}\) cells refractory to TLR9 cell-mediated suppression (42). In addition, dendritic cell secretion of the proinflammatory cytokine IL-6 partially degranulation (45). Histamine signaling through the H1 receptor is reported to enhance cell-mediated suppression of tumor clearance. Immunity 27: 635–645.


Disclosures

The authors have no financial interest of interest.

References


