

## CRTH2 is Critical to the Development of Colitis Induced by Dextran Sodium Sulfate (DSS)

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### Abstract

Chemoattractant Receptor-homologous molecule expressed on Th2 cells [CRTH2] is expressed on granulocytes (eosinophils, basophils and neutrophils), Th2 cells and monocytes. CRTH2 has been implicated in the development of cutaneous inflammation as CRTH2<sup>+</sup> CD4<sup>+</sup> T cells and CRTH2<sup>+</sup> eosinophils are increased in the blood of atopic dermatitis patients. CRTH2 is also up-regulated in circulating neutrophils of psoriatic patients. Interestingly, CRTH2 is detected in the mucosa of patients with Inflammatory Bowel Disease (IBD) ulcerative colitis, where the CRTH2 positive cells localize within and adjacent to regions of inflamed mucosa suggesting that CRTH2 may play a role in IBD. However, the exact downstream inflammatory pathways resulting from CRTH2 activation in colitis are not well characterized. To gain a better understanding of the effects of CRTH2 activation in the pathogenesis of colitis, we have generated a small molecule inhibitor against CRTH2 and its potency was first validated in an oxazolone induced contact dermatitis model. We investigated the consequences of inhibiting CRTH2 in the development of IBD using a Dextran Sodium Sulfate (DSS)-induced colitis mouse model. Compared to the vehicle control group, mice treated with a selective CRTH2 antagonist had reduced disease severity as measured by weight loss, as well as serum acute phase protein, haptoglobin. Furthermore, pro-inflammatory cytokine gene expression for TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-17A, and IFN $\gamma$  were reduced in colons of mice that had been treated with the CRTH2 antagonist compared with DSS treated controls that received vehicle. Taken together, our data identify a previously unrecognized role for CRTH2 in the initiation/amplification and/or stabilization of colon inflammation.

**Keywords:** CRTH2 antagonist; DSS induced colitis; Cytokine and Oxazolone induced-skin inflammation

### Introduction

Prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) is the major prostenoid that is released by activated mast cells and elicits its biological actions through the G-protein-coupled receptors including the prostaglandin D1 receptor (DP1), the thromboxane receptor (TP) and Chemoattractant Receptor homologous molecule expressed on Th2 cells (CRTH2), also known as DP2. PGD<sub>2</sub> is critical to the development of allergic diseases including allergic asthma, rhinitis and atopic dermatitis [1,2] through activating and recruiting Th2 cells, including eosinophils and basophils [3-6].

CRTH2 plays a role in several inflammatory pathways. In human, CRTH2 is preferentially expressed on the Th2 cells, eosinophils and basophils that drive chemotactic responses of these cells to the site of inflammation [7]. Interestingly, in mice, CRTH2 is expressed by both Th1 and Th2 cells at similar levels [8,9]. Previous studies indicated that activation of CRTH<sub>2</sub> via PGD<sub>2</sub> leads to the exacerbation of pathology in a mouse model of asthma [10]. The authors found that a moderate CRTH2 and strong TP antagonist called Ramtrobam, reduced eosinophil accumulation into the mouse lung airways [10]. This observation was further confirmed by using a highly selective CRTH2 antagonist [11] which was shown to inhibit airway eosinophilia in a murine asthma model, as well as to reduce goblet cell hyperplasia. Data from gene knockout studies has revealed that a total lack of the CRTH2 gene can prevent the development of a Th2-type allergic response [12]. Additionally, CRTH2 has been implicated in the development of cutaneous inflammation as CRTH2<sup>+</sup> CD4<sup>+</sup> T cells and CRTH2<sup>+</sup> eosinophils are increased in the blood of atopic dermatitis patients [13]. In mice, CRTH2 antagonists has been shown to effectively attenuate

skin inflammation in various contact sensitivity models [14-16] including the oxazolone induced contact dermatitis model described in this report.

As mucosal immunity involved in the maintenance of barrier function at exposed surfaces (skin, lung and gut) share many similar features, it is not surprising that an increase in the number of CRTH2 expressing cells was detected in the mucosa of IBD patients with ulcerative colitis [17]. This implies that CRTH2 may play a role in the development of colitis. It has been shown that mice receiving DSS orally developed acute and chronic colitis resembling ulcerative colitis [18]. During the acute phase, mice developed colonic mucosal inflammation with ulcerations, body weight loss and bloody diarrhea [18]. To gain an understanding of the potential impact of CRTH2 in the pathology of colitis, we have generated and validated a potent and highly selective CRTH2 inhibitor. We investigated the impact of this selective CRTH2 antagonist in a mouse colitis model induced by Dextran Sodium Sulfate (DSS) [19].

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## Materials and Methods

### Experimental animals

Female, 6-8-week-old BALB/c or C57BL/6 mice (Jackson laboratory, Maine MA) were used in this study. The animals were housed in a vivarium with controlled temperature and light and dark cycles. Mice were fed standard mouse chow pellets and had access to drinking tap water supplied in bottles. This animal protocol was approved by the Institutional Animal Care and Use Committee, Pfizer Inc.

### Reagents

Cyclosporine and DSS, molecular weight 30,000–40,000 was purchased from, MP BioMedicals, Solon, OH, USA. The selective CRTH2 antagonist was generated internally at Pfizer Worldwide Research and Development and is labeled as Compound A in this study.

### Homogeneous time resolved fluorescence assay (FRET) for human CRTH2

The cAMP TR-FRET assay was performed by incubating a dilution series of compound concentrations with 30,000 cells per assay well of CHO-K1 cells expressing the recombinant human CRTH2 receptor (Euroscreen, Belgium). Incubations were performed at room temperature for 30 minutes in the presence of 10  $\mu$ M forskolin and 10 nM PGD<sub>2</sub>. The assay buffer used contained 25 mM HEPES, pH 7.4, 124 mM NaCl, 5 mM KCl, 1.45 mM CaCl<sub>2</sub>, 1.25 mM MgCl<sub>2</sub>, 1.25 mM KH<sub>2</sub>PO<sub>4</sub>, 13.3 mM glucose and 0.5 g/L BSA. Following the 30 minute incubation at room temperature, d2-cAMP and Eu-labeled antibody supplied with the Hi-Range Assay Reagent kit (Cisbio, Bedford, Massachusetts) were separately diluted into the supplier-provided cell lyses buffer, and each was added to the assay plate, as per manufacturer's directions. Data was collected on the Envision plate reader (Perkin Elmer, Waltham, Massachusetts) using  $\lambda$ ex: 340 nm and  $\lambda$ em: 615/665. Samples containing cells and DMSO alone were included as a control in order to measure the maximal signal.

### Homogeneous time resolved fluorescence assay (FRET) for human DP1

The cAMP TR-FRET assay was performed similarly to the CRTH2 assay above but in the absence of forskolin and with the following alterations. Target cells, the NK-92 cell line (ATCC), were maintained according to the vendor and incubated 15 minutes prior to use in assay buffer containing the non-specific phosphodiesterase inhibitor 3-Isobutyl-1-methylxanthine (IBMX, Sigma). 50,000 cells per well were assayed as above in 384 well plates. PGD<sub>2</sub> has an EC<sub>50</sub> of ~100 nM in our hands in this assay for cAMP induction. Test compound was serially diluted and added to cells in the presence of 500 nM PGD<sub>2</sub> for 15 minutes. cAMP levels were then assayed as above. The DP1 selective antagonist A686C (Cayman Chemical), was used as a positive control in this assay and produced a mean IC<sub>50</sub> value of 5.4 nM.

### Human eosinophil shape change assay

Peripheral blood from healthy human donors, prescreened for having greater than 3% eosinophils, was collected into sodium heparin tubes. Blood was incubated with Compound A for 10 minutes at room temperature, then activated with PGD<sub>2</sub> for 10 minutes at 37°C. The reaction was stopped by transferring the plates to ice and adding

1% paraformaldehyde fixative. The samples were then transferred to ammonium chloride lysis solution, mixed gently, and incubated on ice for 40 minutes. Eosinophil shape change was analyzed by flow cytometry using a FACS Caliber outfitted with a 96 well autosampler. Individual cell populations were separated based on their forward and side scatter properties. Granulocytes were gated and assessed for autofluorescence in the FL1 and FL2 channels. Eosinophils were isolated based on their higher autofluorescence in both channels. Finally shape change was measured by alterations in the mean forward scatter of the eosinophils in response to PGD<sub>2</sub>.

### Human basophil chemotaxis assay

Hematopoietic progenitor cells were isolated from peripheral blood of healthy human donors by positively selecting for CD34<sup>+</sup> cells using Miltenyi beads. Cells were grown for 19 days in 10ng/ml recombinant human IL-3 to promote differentiation to CRTH2<sup>+</sup> basophils. Chemotaxis was measured in Corning 96-transwell plates. PGD<sub>2</sub> was included in the bottom well to promote chemotaxis of cells through the 5 $\mu$ M membrane. Cells were pre-incubated with compound for 10 minutes at room temperature before being added to the top of the transwell membrane. An equivalent amount of compound was also included in the bottom well so that no compound gradient would develop. After addition of cells, plates were incubated for 2 hours at 37°C. At the end of the assay, transwell inserts were removed and cells that had migrated to the bottom well were fixed, permeabilized, and labeled using formalin, triton X-100, and hoescht stain. Nuclei were then counted using a Cellomics Arrayscan instrument.

### Competitive radio-ligand binding assay for human CRTH2

Compound inhibition was evaluated using an assay that measures the binding of <sup>3</sup>H-labeled PGD<sub>2</sub> to cells expressing recombinant human CRTH2 receptor. CHO-K1 cells expressing recombinant human CRTH2 (Euroscreen, Belgium) were harvested, washed twice in PBS, and re-suspended in PBS to a cell density of 2 million cells per mL. A dilution series of inhibitor concentrations was prepared in a solution containing 20 nM of [5,6,8,9,12,14,15-<sup>3</sup>H]-PGD<sub>2</sub> (Perkin Elmer, Waltham, Massachusetts, 100-210 Ci/mmol stock) in PBS. The assay was initiated by transferring 50  $\mu$ L of the compound dilution series and 50  $\mu$ L of the re-suspended cells to a 96-well assay plate, for a final assay volume of 100  $\mu$ L, and a final cell density of 100,000 cells per well. Samples containing cells and DMSO alone were included as a control in order to obtain the maximal signal. The assay plate was incubated on ice for 30 minutes, and the cells were harvested onto a Filtermat A (Perkin Elmer, Waltham, Massachusetts) using a Mach 3 Cell Harvester (Tomtec, Hamden, Connecticut). Each harvested Filtermat was enclosed in a Microbeta sample bag (Perkin Elmer, Waltham, Massachusetts), and the sample bag was thermally sealed after scintillation fluid was added. The amount of <sup>3</sup>H bound on the filtermat was counted using a Wallac 1450 Microbeta Trilux (Perkin Elmer, Waltham, Massachusetts).

### Competitive radio-ligand binding assay for the human TP receptor, TBXA<sub>2</sub>R

Inhibitor selectivity was evaluated in an assay that measures the binding of <sup>3</sup>H-labeled ligand to an HEK293 cell line expressing the recombinant human TXA<sub>2</sub>R receptor. The TXA<sub>2</sub>R radio-ligand binding assay is performed in a manner identical to that described for the human CRTH2 receptor, with the exception of the <sup>3</sup>H-labeled ligand.

The TXA<sub>2</sub>R ligand, [5,6-<sup>3</sup>H]-SQ-29548 (Perkin Elmer, 0.1 mCi/mL stock), was used at a final concentration of 10 nM in the assay.

### In-vivo treatment of compound A

A series of concentrations of compound A is usually tested in each model. The dose was decided based on our *in vitro* potency data and *in vivo* modeling results. For acute contact hypersensitivity model the dosage used were 0.3 mg/kg-3 mg/kg P.O and 30 mg/kg for topical. For DSS induced colitis model 10 mg/kg-40 mg/kg of compound A were administered.

### Oxazolone-induced contact dermatitis

In the Oxazolone model of atopic dermatitis, inflammation was measured in the ear skin of mice. One week prior to challenge, mice were sensitized on the shaved abdomen by applying 100 µl of 2% Oxazolone made in 100% ethanol. Five days later, baseline ear thickness measurements were taken on the left and right ears using a Mitutoyo Micrometer (Grainger, Boston, MA). In our studies, mice received either vehicle, Clobetasol or CRTH2 Compound A one hour orally (200 µl) prior to challenge. One hour after treatment, the left ears of the mice were challenged topically with 10 µl of 2% Oxazolone on the left ear, and as a negative control 10 µl of 100% ethanol on the right ear. Mice received either Clobetasol or Compound A orally again as indicated in the figure legends seven hours post challenge. With topical administration, Clobetasol or Compound A were applied 45 minutes prior to and 4 hours after oxazolone application on mice. Measurements were taken with a micrometer at 24 hours post challenge. Ear swelling was expressed as the change (Δ) in ear thickness from pre-challenge baseline values.

### Induction of colitis and treatments

The acute DSS is an experimental UC model that shows the immunological response based on innate mucosal immunity. Acute colitis was induced in mice by feeding with one cycle (5 days) *ad libitum* 3% DSS, molecular weight 30,000–40,000 (MP BioMedicals, Solon, OH, USA) in autoclaved drinking water followed by 8 days of regular drinking water. The DSS solution was changed every 2 days.

Mice were treated orally with either Cremophore vehicle (200 µl) or Cyclosporin A dissolved in Cremophore (15 mg/kg) or methylcellulose and tween 80 with or with various concentrations of Compound A as indicated in the figure legends.

### Histopathological analysis

Colon and skin tissues were collected at necropsy, fixed in formalin, and processed into paraffin tissue blocks using routine methods. The sections were stained with hematoxylin and eosin (H&E). Colon and skin evaluation was performed in a blinded fashion using light microscopy by board certified Veterinary Anatomic Pathologists using a semi- quantitative histopathologic scoring system.

### Haptoglobin detection

Haptoglobin is an acute phase protein induced by various inflammation processes [20]. Serum haptoglobin levels were determined using haptoglobin ELISA kits (R&D Systems) according to manufacturer's instructions.

### Serum cytokine detection

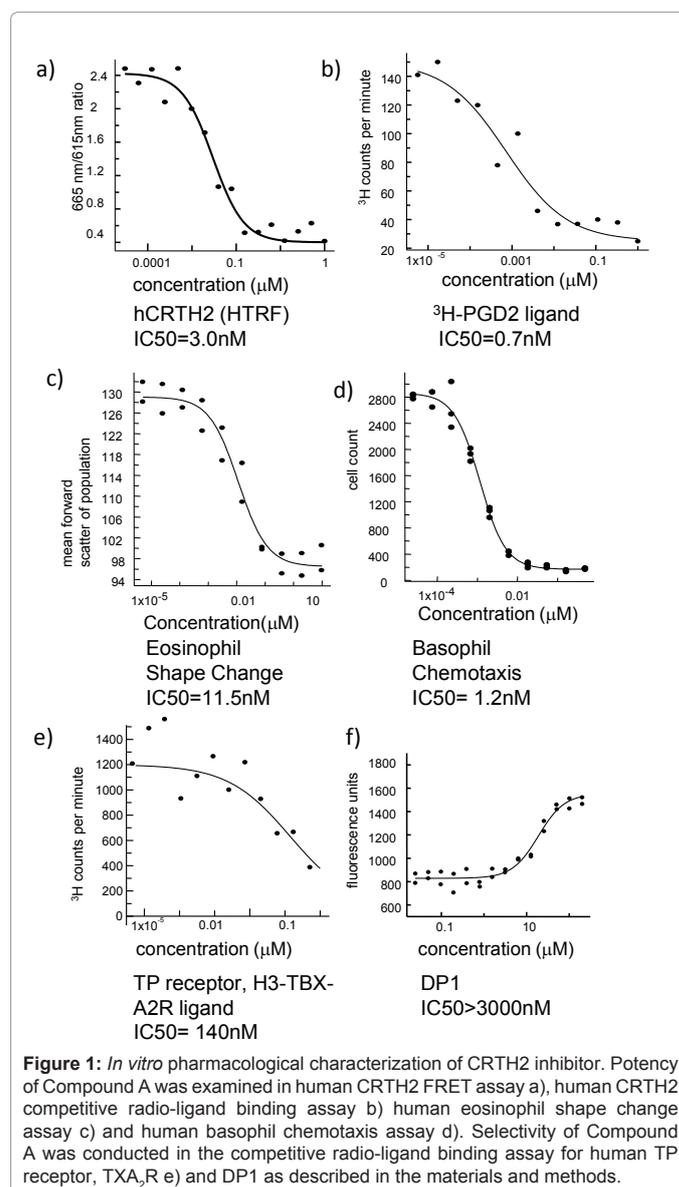
Mouse serum was collected by cardiac puncture at study termination. Serum cytokines were determined using Milliplex Map Kits [Millipore] or Elisa Kits from R&D [R&D Systems] according to manufacturers' instructions.

### Quantitation of cytokine transcripts

RNA was isolated from mouse ear or colon biopsies using the QIAGEN RNeasy kit (QIAGEN). Quantitative RT-PCR for cytokine transcripts was performed using prequalified primers and probes to IL-1β, IL-4, IL-6, IL-12α, IL-13, IL-17A, IL-33, TNF-α, IFN-γ (Applied Biosystems). The ΔCt method was used to normalize transcripts to GAPDH.

### Statistics

Two-tailed Student's t test was used to calculate statistical



significance for differences between groups.  $P \leq 0.05$  was considered statistically significant.

## Results

### Potency and selectivity of CRTH2 inhibitor (Compound A)

*In vitro* pharmacological characterization of Compound A used in these studies is shown in Figure 1. Compound A displayed low nM potency as an antagonist of human CRTH2 in the human CRTH2 FRET assay (Figure 1a) and competitive radio-ligand binding assay (Figure 1b). This compound also demonstrated potency in the primary human eosinophil shape change and basophil chemotaxis assays (Figure 1c and 1d). The selectivity on other closely related PGD<sub>2</sub> receptors TP receptor and DP<sub>1</sub> are remarkable (Figures 1e and 1f, IC<sub>50</sub>=140 nM and >3000 nM respectively). These results suggest that Compound A is a potent and selective CRTH2 antagonist having no discernable activity towards other prostanoid receptors DP<sub>1</sub> and TP receptor (TBX-A2R).

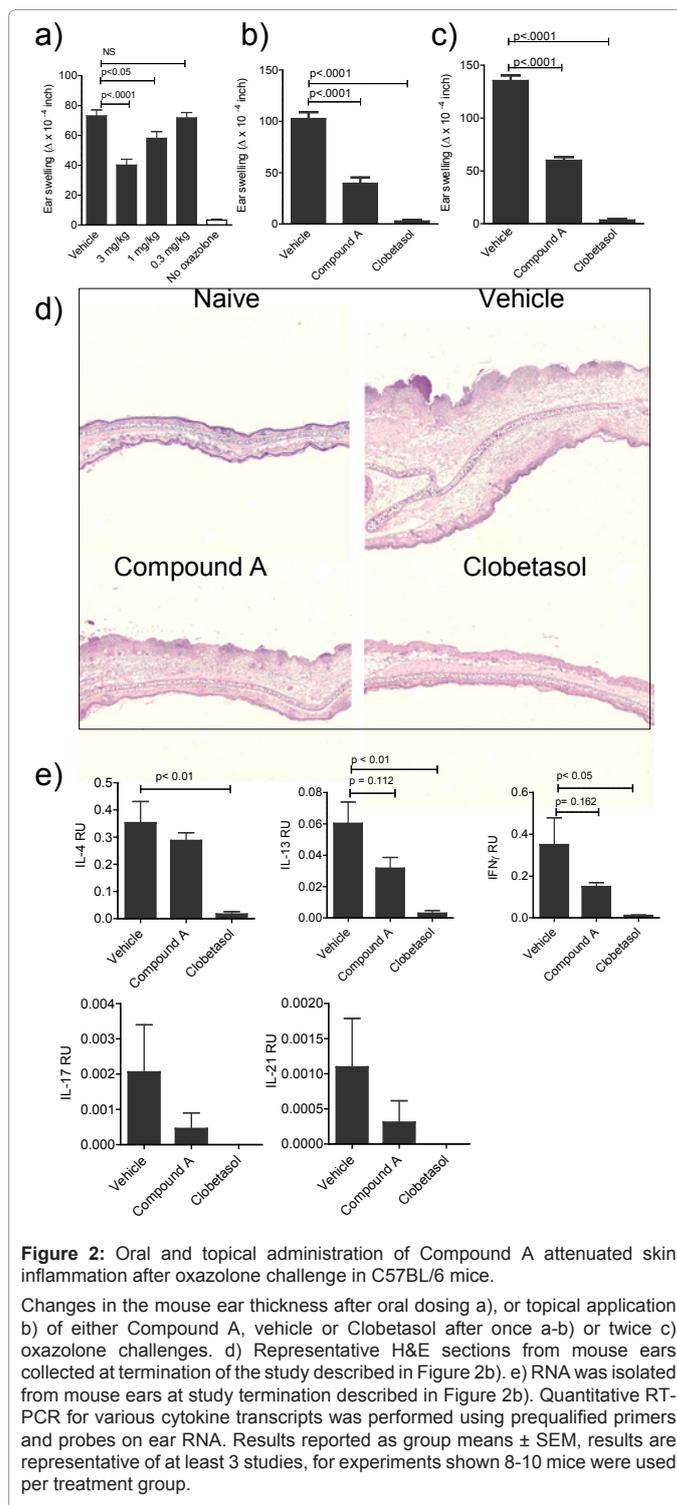
### Compound A prevented disease progression in an oxazolone-induced skin inflammation model

To validate the *in vivo* activity of this CRTH2 antagonist, we evaluated the efficacy of Compound A in the oxazolone-induced acute contact hypersensitivity (CHS) model. In the acute CHS, mice were first topically sensitized on the abdomen with oxazolone. 5 days later, mice were challenged with oxazolone onto the ear. Mice were dosed orally twice daily one hour prior and 7 hours post oxazolone challenge with various concentrations of Compound A. Ear thickness was monitored at 24 hours post oxazolone challenge. Compared to the vehicle treated group, mice receiving Compound A demonstrated improvement in ear swelling in a dose dependent manner (from 0.3 mg/kg to 3 mg/kg) with 3 mg/kg of the compound significantly reducing ear swelling by 40% (Figure 2a).

In the same model, topical application of Compound A (30 mg/kg) 1 hour prior to oxazolone challenge and 4 hours post oxazolone challenge led to remarkable (more than 60%) reduction in ear swelling when compared to the oxazolone challenged ear from vehicle treated mice (Figure 2b). Histopathological analysis revealed that Compound A effectively reduced epidermal thickness, inflammatory infiltrates and edema (Figure 2d) when compared to oxazolone-challenged mice receiving vehicle. Topical application of Compound A also prevented ear swelling (55% reduction in ear thickness) in a repeated oxazolone challenge where oxazolone was applied one week apart (Figure 2c). Clobetasol treatment almost completely prevented ear inflammation in this model, consistent with previous reports [21] (Figure 2 b-d). Interestingly, gene expression analysis indicated that oxazolone challenge induced a substantial up-regulation of cytokine genes including IFN $\alpha$ , IL21, IL-13 and IL-17 in the RNA samples from mice treated with vehicle. These genes were down modulated by Compound A. As expected, mice treated with Clobetasol experienced even more remarkable reduction in expression of these cytokines. Overall, results here are consistent with the previously published results suggesting that the CRTH2 pathway is critically involved in oxazolone-induced CHS. Compound A is efficacious in blocking the CRTH2 pathway and preventing skin inflammation [12,14].

### Cyclosporin A attenuated disease progression in DSS-induced colitis model

To investigate the potential involvement of CRTH2 pathway in the context of IBD colitis, we employed the DSS induced-colitis



**Figure 2:** Oral and topical administration of Compound A attenuated skin inflammation after oxazolone challenge in C57BL/6 mice.

Changes in the mouse ear thickness after oral dosing a), or topical application b) of either Compound A, vehicle or Clobetasol after once a-b) or twice c) oxazolone challenges. d) Representative H&E sections from mouse ears collected at termination of the study described in Figure 2b). e) RNA was isolated from mouse ears at study termination described in Figure 2b). Quantitative RT-PCR for various cytokine transcripts was performed using prequalified primers and probes on ear RNA. Results reported as group means  $\pm$  SEM, results are representative of at least 3 studies, for experiments shown 8-10 mice were used per treatment group.

mouse model. This acute model of colitis is characterized by bloody diarrhea, ulceration and infiltrations with granulocytes that mimic aspects of ulcerative colitis [22,23]. Colitis was induced in C57BL/6 mice as described in the materials and methods. The establishment of this model was first validated with Cyclosporine A treatment shown in Figure 3. DSS-treated mice receiving Cyclosporin experienced significant reduction in percentage weight loss compared to those

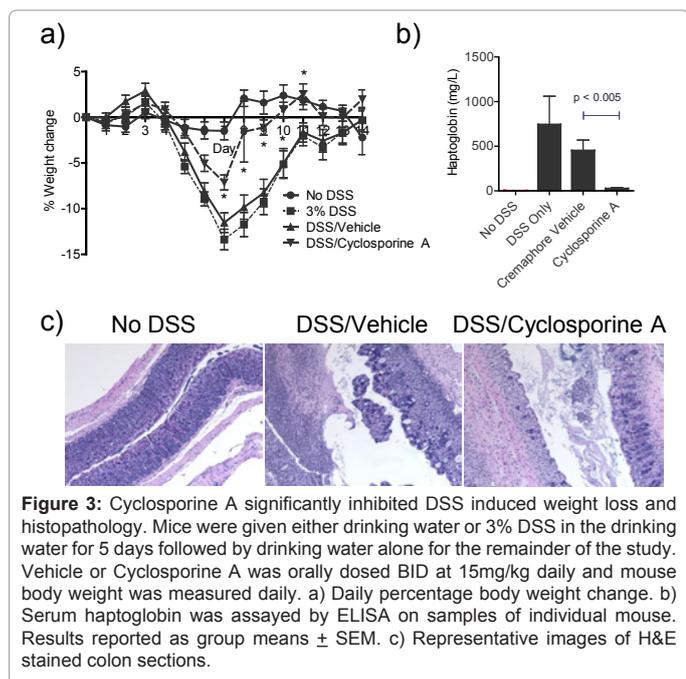
receiving vehicle alone as early as 6 days post Cyclosporin A treatment (Figure 3a). Additionally, mice treated with Cyclosporin A had more than 90% decrease in serum haptoglobin levels (average  $26.1 \pm 4.1$  mg/L) compared to the group receiving vehicle alone [averaged serum concentration  $452.3 \pm 76.3$ mg/L] measured at the end of the study (Figure 3b). Histopathology also confirmed the clinical findings observed after Cyclosporin A treatment. Treated mice displayed reduced amount of colonic inflammatory infiltrates, epithelial hyperplasia and crypt loss (Figure 3c), which is consistent with previously published results [24].

### Compound A prevented disease progression in DSS-induced colitis model

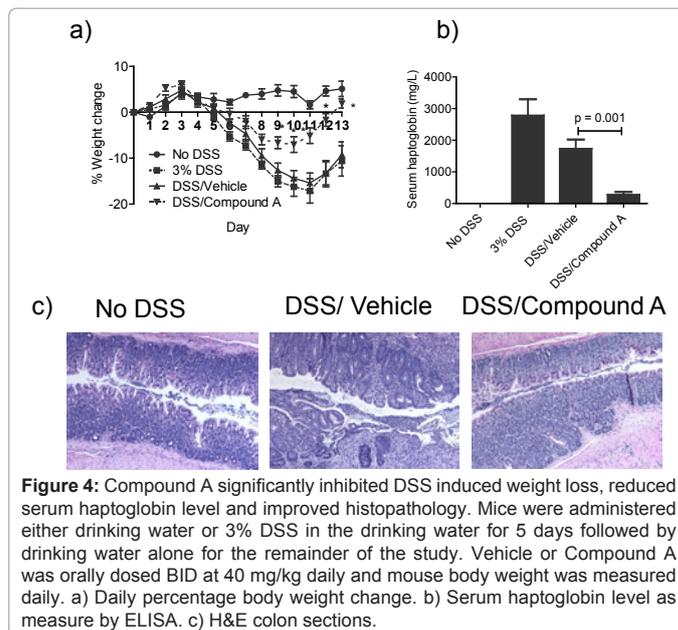
To determine the effect of blocking the CRTH2 pathway in this model, mice were administered daily (10 mg/kg, 20 mg/kg data not shown and) 40 mg/kg of Compound A orally for 14 days. Compared to mice receiving vehicle alone, Compound A-treated mice showed decreased disease severity as measured by percentage weight loss (Figure 4a). At doses of 10 and 20 mg/kg our CRTH2 antagonist demonstrated a trend towards improvement, whereas at 40 mg/kg there was a significant reduction in % weight loss starting on day 9 post initial DSS administration. Furthermore, compared with the vehicle treated group, serum haptoglobin levels were significantly decreased in mice treated with Compound A at 40 mg/kg (Figure 4b). Histopathologically, there was a trend towards improvement in inflammation, hyperplasia, ulceration, crypt loss and fibrosis in colonic sections (Figure 4c).

### Compound A reduced proinflammatory cytokine expression and secretion

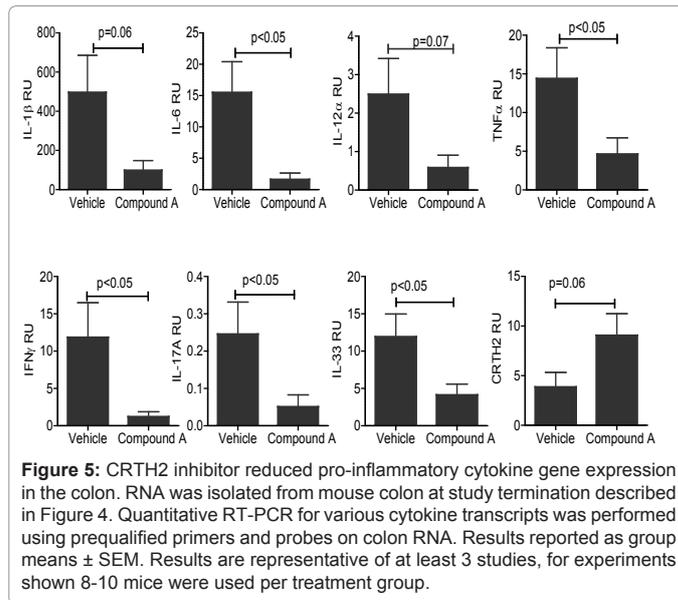
To further elucidate potential mechanisms that may contribute to the function of Compound A in this model, we examined the effects of Compound A on gene expression of various cytokines measured by real time RT-PCR. Relative to vehicle-treated animals, mice treated with Compound A experienced decreased levels of pro-inflammatory genes including IL-12 $\alpha$ , IL-1 $\beta$ , IL-6, IL-17, IFN $\gamma$ , IL-33 (Figure 5).



**Figure 3:** Cyclosporine A significantly inhibited DSS induced weight loss and histopathology. Mice were given either drinking water or 3% DSS in the drinking water for 5 days followed by drinking water alone for the remainder of the study. Vehicle or Cyclosporine A was orally dosed BID at 15mg/kg daily and mouse body weight was measured daily. a) Daily percentage body weight change. b) Serum haptoglobin was assayed by ELISA on samples of individual mouse. Results reported as group means  $\pm$  SEM. c) Representative images of H&E stained colon sections.



**Figure 4:** Compound A significantly inhibited DSS induced weight loss, reduced serum haptoglobin level and improved histopathology. Mice were administered either drinking water or 3% DSS in the drinking water for 5 days followed by drinking water alone for the remainder of the study. Vehicle or Compound A was orally dosed BID at 40 mg/kg daily and mouse body weight was measured daily. a) Daily percentage body weight change. b) Serum haptoglobin level as measure by ELISA. c) H&E colon sections.



**Figure 5:** CRTH2 inhibitor reduced pro-inflammatory cytokine gene expression in the colon. RNA was isolated from mouse colon at study termination described in Figure 4. Quantitative RT-PCR for various cytokine transcripts was performed using prequalified primers and probes on colon RNA. Results reported as group means  $\pm$  SEM. Results are representative of at least 3 studies, for experiments shown 8-10 mice were used per treatment group.

Nevertheless, we also noticed an elevated level of CRTH2. The increase in the CRTH2 gene expression may be due to a negative feedback effect induced by blocking the CRTH2 pathway. Although, not significant, these mice also demonstrated a trend towards decreased IL-12 $\alpha$ , IL-1 $\beta$ , IL-6, IL-17, IFN $\gamma$ , IL-23 and MP1 $\beta$  proteins in the serum (Figure 6) suggesting that CRTH2 pathway blockade may have directly or indirectly suppressed pro-inflammatory gene expression locally and systemically, contributing to improved disease severity.

### Discussion

We report here a highly CRTH2 selective and potent antagonist based on CRTH2 binding, human basophil, and eosinophil cell based assays. Compound A is also very selective against TP and DP $_1$  receptors (Figure 1). Due to its specificity, we believe this compound is a useful tool to analyze the biology of CRTH2 in animal models of

inflammation. Consistent with the role of CRTH2 in promoting allergic inflammation at barrier surface of the skin and lung [14,25,26], CRTH2 inhibitor suppressed the oxazolone-induced contact dermatitis when administered orally or topically by modulating the expression of not only the Th2 cytokine IL13 but also other pro-inflammatory cytokines including IFN $\gamma$ , IL-17 A and IL-21. More importantly, Compound A also reduced the severity of DSS-induced colitis as revealed by improved percentage body weight change, inflammatory biomarker, serum haptoglobin as well as histopathology and cytokine gene/protein expression.

Previous studies with Ramatroban, which was originally identified as a TP antagonist but is also known to bind to the CRTH2 receptor with approximately ten-fold less potent activity, has shown that CRTH2 is important in mediating eosinophil accumulation in a number of tissues in response to an allergic challenge including guinea pig nasal mucosa, mouse airways and in mouse skin during contact hypersensitivity reactions [27]. The effects of Ramatroban on eosinophil recruitment are unlikely to be mediated by TP antagonism as eosinophils do not express TP and selective TP antagonists do not influence eosinophil function [16,28]. In recent studies with highly CRTH2 selective inhibitors, Boehme et al. and Lukacs et al. demonstrated that blocking the CRTH2 effectively prevented allergen induced skin inflammation and airway hyper-responsiveness [25,26]. As expected, they found that CRTH2 blockade remarkably reduced antigen specific serum IgE, IgG1 and IgG2a, and Th2 cytokines IL-13, IL-5. Unexpectedly, similar to our findings, CRTH2 antagonists also prevented expression of acute phase pro-inflammatory cytokines including IL-1 $\beta$ , IL-6, TNF $\alpha$ , Th1 cytokine IFN $\gamma$  and Th17 cytokine IL-17A [25].

Due to the short duration of the oxazolone- and DSS-induced inflammation models, the immune response generated in these models consisted of a mixed cytokine profile including the acute phase pro-inflammatory cytokines (IL-1 $\beta$  and IL-6), Th1/17 (IFN $\gamma$ /IL-17) and Th2 cytokine (IL-13) (Figure 2e and Figure 5) and yet interfering with the CRTH2 pathway has demonstrated efficacy. These results imply that CRTH2 inhibition is exerting a broader immune suppressive effect that is not limited to Th2 cells. This might be related to the receptor-

expression pattern of CRTH2 in mice. Unlike humans in which CRTH2 is expressed selectively by TH2 cells and not TH1 cells, in mice CRTH2 is expressed in equal abundance by both TH1 and TH2 cells [8,9].

In addition to the commonly known impact on Th2 cell activation, CRTH2 inhibition has been shown to affect the activation and trafficking of dendritic cells (DCs) [14]. In particular, studies have shown that human monocytes and mature DCs express CRTH2, and that PGD<sub>2</sub> exerts in vitro chemotactic effects on these cells via CRTH2 activation [29]. In the chronic model of Fluorescein isothiocyanate (FITC) induced contact dermatitis, the authors demonstrated that a CRTH2 antagonist effectively prevented antigen bearing DCs from migrating from skin to the draining lymph nodes, and also reduced the capacity of these DCs to stimulate T cells and cytokine production by the T cells [29]. DCs play critical roles in the pathogenesis of skin inflammation in the oxazolone induced CHS [30] and colon inflammation in the DSS induced colitis [31] respectively. We speculate that Compound A in our studies may also actively inhibit the migration and function of DCs which will prevent the optimal activation T cells to induce inflammation. Additionally, Compound A may also directly inhibit the activity of Th1/Th17 cells in this model.

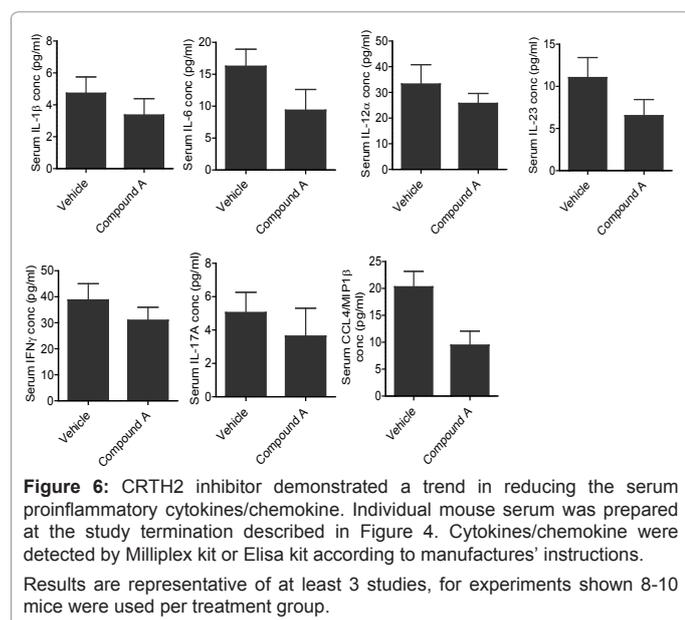
Taken together, we have shown that CRTH2 antagonism is efficacious in attenuating oxazolone induced contact dermatitis and has a profound ability to suppress DSS-induced colitis. Improvement in percentage weight loss in mice treated with CRTH2 antagonist mirrored the clear reduction in inflammatory infiltrates and hyperplasia of the colon (Figure 4). Additionally, the ability of Compound A to suppress variable proinflammatory cytokines involved in the Th1 and Th17 pathways were previously unrecognized in this model, which may warrant further investigation to understand the underlying mechanisms. Nevertheless, our findings support the notion that PGD<sub>2</sub> and CRTH2 engagement may be actively involved in human IBD in particular ulcerative colitis and that inhibiting this pathway may lead to an alternative treatment.

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