EXTENDED REPORT

The histamine H₄ receptor mediates inflammation and Th17 responses in preclinical models of arthritis

Jeffery M Cowden,¹,² Fugu Yu,¹ Homayon Banie,¹ Mandana Farahani,¹ Ping Ling,¹ Steven Nguyen,¹ Jason P Riley,¹ Mai Zhang,¹,³ Jian Zhu,¹ Paul J Dunford,¹ Robin L Thurmond¹

ABSTRACT
Objective The histamine H₄ receptor (H₄R) has been shown to drive inflammatory responses in models of asthma, colitis and dermatitis, and in these models it appears to affect both innate and adaptive immune responses. In this study, we used both H₄R-deficient mice and a specific H₄R antagonist, JNJ 28307474, to investigate the involvement of the H₄R in mouse arthritis models.

Methods H₄R-deficient mice and wild-type mice administered the H₄R antagonist were studied in models of collagen antibody-induced arthritis (CAIA) and collagen-induced arthritis (CIA). The impact on Th17 cells was assessed by restimulation of inguinal lymphocytes in the disease or immunisation models and with in vitro stimulation of whole blood.

Results Both H₄R-deficient mice and mice treated with the H₄R antagonist exhibited reduced arthritis disease severity in both CAIA and CIA models. This was evident from the reduction in disease score and in joint histology. In the CIA model, treatment with the H₄R antagonist reduced the number of interleukin (IL)-17 positive cells in the lymph node and the total production of IL-17. Th17 cell development in vivo was reduced in H₄R-deficient mice or in mice treated with an H₄R antagonist. Finally, treatment of both mouse and human blood with an H₄R antagonist reduced the production of IL-17 when cells were stimulated in vitro.

Conclusions These results implicate the H₄R in disease progression in arthritis and in the production of IL-17 from Th17 cells. This work supports future clinical exploration of H₄R antagonists for the treatment of rheumatoid arthritis.

INTRODUCTION
The histamine H₄ receptor (H₄R) has been linked to inflammation in several preclinical models and it holds promise as a target for treating allergic inflammation (for a recent review, see Walter et al¹). Not so obvious would be a role in autoimmune diseases, although changes in histamine levels have been observed in such conditions.²⁻⁴ In addition, H₄R expression has been found in the synovial cells, primarily on fibroblast-like and macrophage-like cells, from patients with rheumatoid arthritis.⁵ ⁶

Most of the models showing a role for the H₄R in inflammation are allergic or Th2-driven inflammation, which is commonly associated with histamine involvement. However, the H₄R has been shown to mediate T cell responses in humans and mice.⁷⁻¹¹ Indeed in the mouse asthma model, mice treated with an H₄R antagonist only during the sensitisation phase of the model, where T cell responses are initiated, exhibit reduced disease.⁷⁻¹² The effect on T cells has prompted the question as to whether the H₄R has roles beyond Th2-driven inflammation and whether the receptor could be involved in autoimmune diseases.¹³ The receptor has been shown to be expressed on human Th17 cells, and in these cells can mediate the production of interleukin (IL)-17.¹⁴ Consistent with this, H₄R-dependent decreases in IL-17 have been consistently shown even in mouse Th2-driven inflammation models.⁷ ⁹

In this work the requirement for the H₄R is shown in both a mouse collagen-induced (CIA) and a collagen antibody-induced arthritis (CAIA) model. Having effects in both models suggests a role for the H₄R in both innate and adaptive immune responses that drive arthritis in humans. In particular, one of the underlying mechanisms for the H₄R effects may be in part due to modulation of Th17 cells. These results suggest that antagonism of the H₄R is promising target for treating autoimmune diseases such as rheumatoid arthritis.

METHODS
Arthritis models
For the CAIA model, BALB/c mice were given 2 mg collagen antibody cocktail (Chondrex, Redmond, Washington, USA) intravenously on day 1 and then challenged with 20 μg lipopolysaccharide (LPS) by intraperitoneal injection on day 3. Disease onset occurred on day 4, and mice were examined visually daily for the appearance of arthritis in the peripheral joints. For the CIA model, DBA1/J mice were injected at the base of the tail with bovine type II collagen (Chondrex) emulsified in complete Freund’s adjuvant (CFA) per the manufacturer’s protocol. On day 26, mice received 20 μg LPS by intraperitoneal injection to synchronise the onset of arthritis. Animals were enrolled into treatment groups on days 27–28 when any paw had a score of 1 or greater. To induce arthritis in C57BL/6 H₄R-deficient and wild-type animals, the method was modified to include two CFA/collagen injections similar to that described previously.¹⁵ For all models, the severity of arthritis was graded on a scale of 0–4 for each paw in a blinded fashion. The scores for each of the four paws were added
together to give a final score such that the maximal severity score was 16, which is presented as mean±SEM. Where applicable, mice were treated orally (by gavage) with vehicle or the H4R antagonist, JNJ 28307474, at the indicated doses twice a day at the time of disease onset (defined as a score of 1 or greater in any paw). Paw tissue was prepared, and histological analyses were performed as previously described. In addition, inguinal lymph nodes were collected in some studies and pooled per treatment group. A single-cell suspension (RPMI 1640 supplemented with 10% fetal bovine serum, non-essential amino acids and 2-mercaptoethanol) was prepared, and triplicates (10^5 cells/well) were plated in a 96-well plate coated with 2 μg/mL anti-CD3 and 1 μg/mL anti-CD28. After 24 h, supernatants were collected, and IL-17 and interferon (IFN)γ were measured by ELISA. In addition, cells were stained for CD4 and intracellular IL-17 and analysed by fluorescein-activated cell sorting (FACS).

**Th17 cells models**

An adoptive transfer model for Th17 cell development was conducted as previously described. Where indicated, mice were treated with vehicle or JNJ 28307474 (50 mg/kg twice daily) starting the day after transfer of the OT-II cells just before the immunisation. Details on the in vitro Th17 cell experiments are given in the online supplementary material.

**Statistical analysis**

Details on the statistical analysis are given in each figure caption and in the online supplementary material. All statistical analysis was carried out using GraphPad Prism.

More detailed materials and methods are given in the online supplementary material.

**RESULTS**

**CAIA model**

Wild-type and H4R-deficient mice on the BALB/c background were studied in the CAIA model. In wild-type mice, there was an increase in clinical score that peaked and plateaued around day 5 (figure 1A). The same pattern was seen in the H4R-deficient mice, but the disease severity, as judged by the clinical scores, was dramatically decreased. A Wilcoxon ranked sum test indicated a significant difference in the time courses (p<0.01), and there was a statistically significant difference between wild-type and H4R-deficient mice at every time point. When the disease severity is expressed as area under the curve (AUC), a statistically significant reduction in disease severity can be clearly seen (figure 1B). Histological examination was conducted to illustrate the joint pathology in diseased mice (figure 2A). Consistent with the clinical score, the H4R-deficient mice showed a significant reduction in disease pathological severity, as indicated by inflammation, pannus, cartilage damage and bone damage (figure 2B). As mast cells are a potential source of histamine in the synovium, the numbers of mast cells in the inflamed synovial lining of the diseased mice in different fields were counted, and a significant reduction in the mean mast cell numbers was observed between the wild-type and H4R-deficient mice (see online supplementary figure S1A).

The data with the H4R-deficient mice point to a role for the receptor in mediating the inflammation seen in the CAIA model. To confirm this, JNJ 28307474, a potent and specific H4R antagonist with a relatively long half-life in mice, was used. JNJ 28307474 was given orally at various doses twice a day starting at the time of disease onset (any paw with a score of 1 or greater). Treatment with 20 and 50 mg/kg JNJ 28307474 significantly reduced the severity score, as seen both from the time course (p<0.01 for 50 mg/kg by Friedman test) and the AUC of the score (figure 3A, B). The inhibition observed at 50 mg/kg was similar to that seen in the H4R-deficient mice (figure 1B).

The H4R is expressed on several cell types that may be involved in modulating the inflammation in this model. Of particular interest are dendritic cells, since previously it was shown that lack of the H4R on splenic CD11c+ cells impaired their ability to activate T cells in vitro. To investigate the role of these cells in the CAIA model, CD11c+ cells isolated from the spleens of wild-type or H4R-deficient mice were injected into H4R-deficient mice before antibody administration. H4R-deficient mice that received H4R-deficient CD11c+ cells had a reduced severity score compared with wild-type mice (see online supplementary figure S2). However, when these mice...
received wild-type CD11c+ cells, the severity score was similar to that of wild-type mice, suggesting that the H4R on these cells was contributing to the disease progression. The in vivo phenotype of these transferred monocytic cells is unclear and further work is needed to understand their relative contribution to the innate and adaptive components of pathology in this model.

Figure 2 Effects on histology in the collagen antibody-induced arthritis model. (A) Paws were collected for histology and stained with toluidine blue. Representative images are shown for wild-type mice on the left and histamine H4 receptor (H4R)-deficient mice on the right. The top images are a 16× magnification, whereas the bottom ones are 200×. (B) Sections for all animals were scored for inflammation, pannus, cartilage and bone damage, and the mean and SEM of these scores are given. Statistical comparison of wild-type (black bars, n=5) and H4R-deficient (white bars, n=9) mice was conducted using a Mann–Whitney test. *p<0.05, **p<0.01.
therefore the model was adapted to this strain. Before receiving the LPS boost, the incidence of arthritis in the H4R-deficient mice was lower (1/12) than in wild-type mice (10/12), and the average score was significantly reduced (figure 4C). After receiving the LPS boost, all of the wild-type animals (12/12) developed scores of >2, but only 7 of 12 H4R-deficient animals developed disease. At this point, there was no statistically significant difference in the disease scores (figure 4C). Fourteen days later, all of the wild-type mice still exhibited the same level of disease as at day 5, but the disease appeared to improve in the H4R-deficient mice, as evidenced by two mice (final incidence 5/12) completely recovering (score <2) and a statistically significant decrease in the average score starting at day 9 (figure 4C). Furthermore, if wild-type mice were treated with JNJ 28307474 on day 5 after they all developed disease, the mice started to recover, as measured by a decrease in the average disease score (figure 4C), and at day 19 the average disease score was similar to that seen in the H4R-deficient mice. Overall, there was a statistically significant decrease in the incidence of arthritis in the H4R-deficient mice using either a Fisher’s exact test (p<0.007) or a log-rank survival method (p<0.002). In total, these results confirm that the H4R can mediate inflammation in the mouse CIA model.

Histological examination was also conducted in this model to illustrate the joint pathology in diseased mice, and representative data are shown in figure 5A. Scoring of inflammation, pannus, cartilage damage and bone damage showed inhibition by 30 mg/kg JNJ 28307474 for all of these variables (figure 5B). This is consistent with the effects seen with the CAIA model. As for the CAIA model, the mast cell numbers in the joint were decreased with treatment with JNJ 28307474 (see online supplementary figure S1B), and these have been reported to be increased in the model.19 To further support the histological finding of a reduction in joint inflammation, expression of inflammatory cytokines and chemokines in the joint was assessed. The message levels of IL-6, IL-1β, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-2 and tumour necrosis factor (TNF) were all reduced in the joints of animals treated with [JNJ 28307474, although only the reductions in IL-6, MCP-1, MIP-2 reached statistical significance (see online supplementary figure S3). There was no statistically significant reduction in collagen-specific IgG levels (see online supplementary figure S4).

Th17 cell development
In the CIA model, the percentage of IL-17+ CD4 cells in the inguinal lymph node were increased in diseased animals compared with naïve animals, and treatment with [JNJ 28307474 led to a reduction in this percentage (figure 6A). In addition, restimulation of lymphocytes with anti-CD3 and anti-CD28 resulted in the production of IL-17 in diseased animals, and this was reduced when the animals were treated with [JNJ 28307474 (figure 6B). No effect of the H4R antagonist on IFNγ was seen, although it was increased in diseased animals (figure 6B).

It is difficult to determine whether the reduction in Th17 cells seen with H4R antagonist treatment in CIA is due to a direct role of the H4R in Th17 cell function or whether this only reflects a reduction in the inflammation driven by other anti-inflammatory mechanisms. Therefore, the role of the H4R on Th17 cell development in vivo was directly assessed using an adoptive transfer model with transgenic OT-II T cells specific for ovalbumin.17 Treatment with JNJ 28307474 led to a reduction in the number of OT-II Th17+ cells in the lymph node (figure 6C). A role for the H4R in Th17 cell development in vivo was confirmed using H4R-deficient mice. Transferring

Figure 3 Effects of JNJ 28307474 in the collagen antibody-induced arthritis model. (A) Wild-type BALBc mice (n=5 per group) were given collagen antibody cocktail and then challenged with lipopolysaccharide (LPS) by intraperitoneal injection 2 days later. The day after LPS administration, mice where treated with vehicle (○), 5 mg/kg (▲), 20 mg/kg (▼) or 50 mg/kg (●) JNJ 28307474. All doses were given orally twice a day. The mice were examined visually for the appearance of arthritis in the peripheral joints, and the severity of arthritis was graded on a scale of 0–4 for each paw. The mean and SEM for the sum of the severity scores are given. (B) The area under the curve (AUC) for each time course was calculated, and the mean AUC and SEM are given. For both panels, statistical significance between each JNJ 28307474 group and vehicle was assessed by one-way analysis of variance with the post hoc Dunnett’s test. *p<0.05, **p<0.01, ***p<0.001.

CIA model
To further understand the role of the H4R in mediating arthritis, a CIA model was used. As for the CAIA model, treatment with JNJ 28307474 led to a dose-dependent reduction in the disease severity score, with the highest dose of 50 mg/kg showing little increase in disease activity over the baseline (figure 4A). The time courses for the 20 and 50 mg/kg doses were significantly reduced compared with the vehicle control (p<0.001 with a Friedman test). Calculation of the AUC of the severity score indicated that there was a trend for reduction at 5 and 20 mg/kg, but a statistically significant reduction at 50 mg/kg (figure 4B).

The reduction in severity score with the H4R antagonist suggests that the receptor mediates inflammation in this model. To confirm this, studies were carried out in H4R-deficient mice. However, the standard model is conducted in DBA1/J mice and the H4R-deficient mice were on the C57BL/6 background, and...
H4R-deficient OT-II cells into wild-type or H4R-deficient mice also led to reduction in the total number of OT-II Th17+ cells as seen with H4R antagonist treatment (figure 6D). In addition, transfer of wild-type OT-II cells into H4R-deficient mice also produced the same effect. These results show that the H4Ro n both T cells and other host cells are necessary for Th17 cell development in vivo.

Recently, H4R expression has been shown on human Th17 cells, and the production of IL-17 is increased by treatment with an H4R agonist.14 Mouse Th17 cells can also express the H4R, as determined by reverse transcriptase-PCR (data not shown), and the impact of the H4R on IL-17 in these cells was explored. Blood stimulated with anti-CD3/CD28 and IL-23 led to an increase in IL-17 production, and this was decreased in blood taken from H4R-deficient mice or mice treated in vivo with the H4R antagonist, JNJ 28307474 (figure 6E).

DISCUSSION
The data presented here support a role for the H4R in arthritis. In a CAIA model, H4R-deficient mice were largely protected from disease, as judged by a reduction in disease score and by joint histology. A very similar effect was seen when mice were treated with the H4R antagonist, JNJ 28307474. The fact that
there are similar effects with H4R-deficient mice and H4R antagonist strongly supports a role for the H4R in this model. These results are similar to those seen in the K/BxN model of arthritis with histidine decarboxylase-deficient mice that lack histamine. The CAIA and K/BxN transfer model have similar underlying mechanisms and therefore it is reasonable to assume that the effects reported in histidine decarboxylase-deficient mice are due to lack of histamine activation of the H4R. Both of these models are thought to be driven by activation of the innate immune system, and T cells are not thought to be involved until later in the disease progression. While the exact mechanisms for the role of the H4R in the models are not known, transfer of wild-type CD11c+ cells can restore the disease in H4R-deficient mice, and there is evidence that the receptor can play a role in mast cell, dendritic cell, NK T cell and macrophage activation. Therefore, accumulating data suggest that H4R is a crucial player in modulating innate cell activation, which is important for initiating inflammatory responses and explains the effects seen in the CAIA model.

To further explore the potential role of the H4R in arthritis, a CIA model was used that has a strong T cell component. As for the inflammatory arthritis model, both H4R-deficient mice and mice treated with an H4R antagonist, JNJ 28307474, exhibited a reduction in severity score and inflammation. H4R antagonist treatment is effective whether the compound is given semi-therapeutically after the mice show the first signs of disease (figure 4A) or therapeutically when animals have the maximum score (figure 4C). Similar effects were observed in H4R-deficient mice. Interestingly, before the LPS boost, H4R-deficient mice had a lower incidence of disease compared with wild-type mice. The administration of LPS led to increased disease in both the wild-type and H4R-deficient animals, although the incidence and average score trended to be lower in the H4R-deficient mice. So it appears that the H4R-deficient mice are protected from developing arthritis in the model, but that some of this can be overcome by adding a strong inflammatory stimulus such as LPS. However, even though the LPS initially tended to increase the disease score in the H4R-deficient mice, after this

---

**Figure 5** Effects on histology in the collagen-induced arthritis model. (A) Paws were collected for histology and stained with H&E (top) and toluidine blue (bottom). Representative images are shown for naive (unimmunised) mice (left), immunised and vehicle-treated (middle) and immunised and treated with 50 mg/kg JNJ 28307474 orally (right). (B) Sections for all animals were scored for inflammation, pannus, cartilage and bone damage, and the mean and SEM of these scores are given. Statistical comparison of treated groups (n=11 per group) with the vehicle group (n=11) was assessed by one-way analysis of variance with post hoc Dunnett’s test. *p<0.05.
point they started to recover, whereas the wild-type mice have stable disease. Consistent with this, wild-type mice treated with JNJ 28307474 after the LPS boost, when they have the maximum score, started to recover and had a similar severity score to the H4R-decient mice on day 19 that was significantly better than that of the wild-type mice.

The effects of the H4R in the CIA model could be at least partly mediated by effects on Th17 cells. It is known that the model is dependent on Th17 cells,25–27 and treatment with the H4R antagonist significantly reduced the number of IL-17+ cells in the lymph node and the secretion of IL-17 when lymphocytes were stimulated ex vivo. This could result from a direct role for the H4R in Th17 cells, since, in an immunisation model, H4R-decient mice or mice treated with an H4R antagonist showed a reduction in the development of Th17 cells (figure 6C,D). Of interest, the effects on the development of Th17 cells were observed in vivo. The effects of the H4R in the CIA model could be at least partly mediated by effects on Th17 cells. It is known that the model is dependent on Th17 cells25–27 and treatment with the H4R antagonist significantly reduced the number of IL-17+ cells in the lymph node and the secretion of IL-17 when lymphocytes were stimulated ex vivo. This could result from a direct role for the H4R in Th17 cells, since, in an immunisation model, H4R-deficient mice or mice treated with an H4R antagonist showed a reduction in the development of Th17 cells (figure 6C,D). Of interest, the effects on the development of Th17 cells were observed in vivo.

**Figure 6** Effects of the histamine H4 receptor (H4R) on Th17 cells. The percentage of interleukin (IL)-17+ CD4 cells in the inguinal lymph node (A) and the levels of IL-17 and interferon (IFN)γ produced after stimulation with anti-CD3/anti-CD28 (B) were measured in naive (unimmunised) mice, vehicle-treated mice or mice treated orally with 50 mg/kg JNJ 28307474 twice daily. (C) OT-II cells were transferred into wild-type (WT) mice 1 day before immunisation with the specific ovalbumin peptide. Starting the day of immunisation, mice were treated with vehicle (n=7) or 50 mg/kg twice daily JNJ 28307474 orally (n=6). Ten days later lymph nodes were harvested and the total number of OT-II Th17+ cells was determined by FACS. (D) WT or H4R-deficient (KO) OT-II cells were transferred into either WT or H4R-deficient (KO) host mice (n=6–8 mice per group). One day later, the mice were immunised with the specific ovalbumin peptide. Ten days later, lymph nodes were harvested and the total number of OT-II Th17+ cells was determined by fluorescein-activated cell sorting. (E) Blood from H4R-deficient mice (H4R−/−), WT mice treated orally for 20 min with 20 mg/kg JNJ 7777120 or vehicle was stimulated in vitro with anti-CD3/CD28 and IL-23 for 18 h. IL-17 measured by ELISA. Non-stimulated (Non-Stim) blood from WT mice was used as a control. (F) Human peripheral blood mononuclear cells (n=5 donors; 2–4 replicates each) were incubated with vehicle (dimethyl sulfoxide, black bars), 1 μM JNJ 7777120 (open bars) or 1 μM JNJ 28307474 (hatched bars) for 1 h and then various stimuli were added as indicated and further incubation for 48 h. IL-17 was measured by ELISA. For (A–C) statistical significance was determined by a Mann-Whitney test. *p<0.05, ***p<0.001, ns is not significant when comparing JNJ 28307474 treatment with vehicle. ψp<0.01 comparing vehicle with naïve animals. For (D–F) statistical significance between groups was assessed by one-way analysis of variance with post hoc Dunnett’s test. *p<0.05, **p<0.01, ***p<0.001.
Th17 cells in the adoptive transfer model were apparent when either the donor T cells or the recipients were H4R-deficient. This implies that the receptor is required on both T cells and antigen-presenting cells for optimal Th17 cell development. The results in mice appear to be consistent with effects on human Th17 cells, where blocking the H4R in vitro inhibits IL-17 production from human peripheral blood mononuclear cells. This is consistent with recent work showing that human Th17 cells express the H4R and that IL-17 production can be increased with an H4R agonist. Therefore, the H4R appears to play a direct role in Th17 activity. This may contribute to the effects seen in the CIA model, but the changes observed for inflammatory mediators such as IL-6 and TNF suggest that other mechanisms may be involved.

One outstanding question is the source of histamine in the animal models and its relevance to human arthritis. It is well-known that mast cells and basophils secrete histamine and thus are potential sources. Mast cells are known to be increased in the CIA model, and here it is shown that treatment with an H4R antagonist in both models reduces the number of mast cells in the synovial lining. Mast cells have been shown to be important mediators in some animal models of arthritis and have been found to be increased in the synovium of patients with rheumatoid arthritis; however, it is still not clear whether they are key players in the disease. Basophils may also play a role, and it was recently shown that histamine release from basophils amplifies IL-17 release from T cells. It is important to note that the H4R has a very high affinity for histamine, especially compared with the histamine H1 and H2 receptors, and therefore it may be activated by cells that release even low levels of histamine. To this point, it has become apparent that many immune cells, such as dendritic cells, T cells, and neutrophils, are capable of producing histamine when stimulated. It has been speculated that local production of histamine by dendritic cells can act in an autocrine fashion to modulate dendritic cell/T cell interactions, and this local production of histamine in the joint or at sites of T cell activation may be the most relevant for H4R activation in human arthritis.

Overall, the data presented show an anti-inflammatory role for H4R antagonists in preclinical models and support the clinical study of such antagonists for the treatment of rheumatoid arthritis. This is further supported by the known safety profile of H4R antagonists. The H4R-deficient mice are fertile and healthy and, outside of effects on inflammatory response, appear to have no other defects. In addition, no safety issues have been observed with compound treatment either in this work or in other animal models. While it is still early days, there have been reports of H4R in phase I clinical studies with no safety issues related to H4R antagonism reported (for a summary, see Salcedo et al). Therefore, H4R antagonists may provide a safe and effective alternative for the treatment of rheumatoid arthritis.

The protocol for the induction of arthritis in healthy and, outside of effects on inflammatory response, was performed by Bolder BioPATH (Boulder, Colorado, USA).

REFERENCES

1. Walter M, Kotte T, Stark H. The histamine H4 receptor: targeting inflammatory disorders. Eur J Pharmacol 2011;668:1–5.
2. Tuomisto L, Kilpelainen H, Riekkinen P. Histamine and histamine-N-methyltransferase in the CSF of patients with multiple sclerosis. Agents Actions 1982;12:255–257.
3. Frewn DB, Cleden LG, Jonsson JR, et al. Histamine levels in human synovial fluid. J Rheumatol 1986;13:13–14.
4. Winterkamp S, Weidenhiller M, Otte P, et al. Urinary excretion of N-methylhistamine as a marker of disease activity in inflammatory bowel disease. Am J Gastroenterol 2002;97:3071–7.
5. Ohki E, Suzuki M, Aze T, et al. Expression of histamine H4 receptor in synovial cells from rheumatoid arthritis patients. Biol Pharm Bull 2007;30:2217–20.
6. Ikawa Y, Suzuki M, Shiono S, et al. Histamine H4 receptor expression in human synovial cells obtained from patients suffering from rheumatoid arthritis. Biol Pharm Bull 2005;28:2016–18.
7. Dunford PL, O’Donnell N, Riley JP, et al. The histamine H2 receptor mediates allergic airway inflammation by regulating the activation of CD4+ T cells. J Immunol 2006;176:7062–70.
8. Cowden JM, Riley JP, Ma JH, et al. Histamine H4 receptor antagonism diminishes existing airway inflammation and dysfunction via modulation of Th2 cytokines. Respir Res 2010;11:86.
9. Cowden JM, Zhang M, Dunford PJ, et al. The histamine H4 receptor mediates inflammation and Pruritus in Th2-dependent dermatitis. J Invest Dermatol 2010;130:1023–33.
10. Lundberg K, Broos S, Greiff L, et al. Histamine H4 receptor antagonism inhibits allergen-specific T-cell responses mediated by human dendritic cells. Eur J Pharmacol 2011;651:197–204.
11. Gutzmer R, Momment S, Gschwandtner M, et al. The histamine H4 receptor is functionally expressed on TH2 cells. J Allergy Clin Immunol 2009;123:619–26.
12. Beermann S, Glage S, Jonik D, et al. Opposite effects of mepyramine on THI 7777120-induced amelioration of experimentally induced asthmic mice in sensitization and provocation. PLoS ONE 2012;7:e30285.
13. Zhang M, Venable JD, Thurmond RL. The histamine H4 receptor in autoimmune disease. Expert Opin Invest Drugs 2006;15:1443–52.
14. Momment S, Gschwandtner M, Koether B, et al. Human memory Th17 cells express a functional histamine H4 receptor. Am J Pathol 2012;180:177–85.
15. Inglis J, Simelyte E, McCann FE, et al. Protocol for the induction of arthritis in C57BL/6 mice. Nat Protoc 2008;3:612–18.
16. Bendele AM, Chilpala ES, Scherer J, et al. Combination benefit of treatment with the cytokine inhibitors interleukin-1 receptor antagonist and PEGylated soluble tumor necrosis factor receptor type I in animal models of rheumatoid arthritis. Arthritis Rheum 2000;43:1649–59.
17. McGreathy MJ, Chen Y, Tato CM, et al. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nat Immunol 2009;10:314–24.
18. Cowden JM, Yu F, Challapalli M, et al. Antagonism of the Histamine H4 receptor reduces LPS-induced TNF production in vivo. Inflammation Res 2013;62:599–607.
Increases in mast cells and chymase in fibroproliferative paws of collagen-induced arthritic mice. *Inflammation Res* 1999;48:318–24.

20 Rajasekaran N, Solomon S, Watanabe T, et al. Histidine decarboxylase but not histamine receptor 1 or 2 deficiency protects from K/BxN serum-induced arthritis. *Int Immunol* 2009;21:1263–8.

21 Mitamura M, Nakano N, Yonekawa T, et al. T cells are involved in the development of arthritis induced by anti-type II collagen antibody. *Int Immunopharmacol* 2007;7:1360–8.

22 Desai P, Thurmond RL. Histamine H4 receptor activation enhances LPS-induced IL-6 production in mast cells via ERK and PI3K activation. *Eur J Immunol* 2011;41:1764–73.

23 Leite-de-Moraes MC, Diem S, Michel M-L, et al. Cutting edge: histamine receptor H4 activation positively regulates in vivo IL-4 and IFN-gamma production by invariant NKT cells. *J Immunol* 2009;182:1233–6.

24 Lubberts E, Koenders MI, Oppers-Walgreen B, et al. Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. *Arthritis Rheum* 2004;50:650–9.

25 Nakae S, Nambu A, Sudo K, et al. Suppression of Immune Induction of Collagen-Induced Arthritis in IL-17-Deficient Mice. *J Immunol* 2003;171:6173–7.

26 Lubberts E, Joosten LAB, Oppers B, et al. IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. *J Immunol* 2001;167:1004–13.

27 Nigrovic PA, Lee DM. Synovial mast cells: role in acute and chronic arthritis. *Immunol Rev* 2007;217:19–37.

28 Suurmond J, Schuvenwegh AIM, Toes REM. Anti-citrullinated protein antibodies in rheumatoid arthritis: a functional role for mast cells and basophils? *Ann Rheum Dis* 2010;70:55–8.

29 Wakahara K, Baba N, Van VO, et al. Human basophils interact with memory T cells to augment Th17 responses. *Blood* 2012;120:4761–71.

30 Alcaniz L, Vega A, Chacon P, et al. Histamine production by human neutrophils. *FASEB J* 2013;27:2902–10.

31 Aoi R, Nakashima I, Kitamura Y, et al. Histamine synthesis by mouse T lymphocytes through induced histidine decarboxylase. *Immunology* 1989;66:219–23.

32 Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. *Nat Rev Drug Discov* 2008;7:41–53.

33 Varga C, Horvath K, Berko A, et al. Inhibitory effects of histamine H4 receptor antagonists on experimental colitis in the rat. *Eur J Pharmacol* 2005;522:130–8.

34 Salcedo C, Pontes C, Merlos M. Is the H4 receptor a new drug target for allergies and asthma? *Front Biosci Elite Ed* 2013;E5:178–87.