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EXTENDED REPORT

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

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Handling editor Tore K Kvien

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/annrheumdis-2013-203832>).

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Received 23 April 2013

Revised 12 August 2013

Accepted 20 September 2013

Published Online First

14 October 2013

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.

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 a 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

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 al*¹). 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



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To cite: Cowden JM, Yu F, Banie H, *et al.* *Ann Rheum Dis* 2014;**73**:600–608.

together to give a final score such that the maximal severity score was 16, which is presented as mean \pm 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.¹⁶ 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 (10⁵ cells/well) were plated in a 96-well plate coated with 2 μ g/mL anti-CD3 and 1 μ g/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.¹⁷ 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 along 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,^{9 18} 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*.⁷ 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

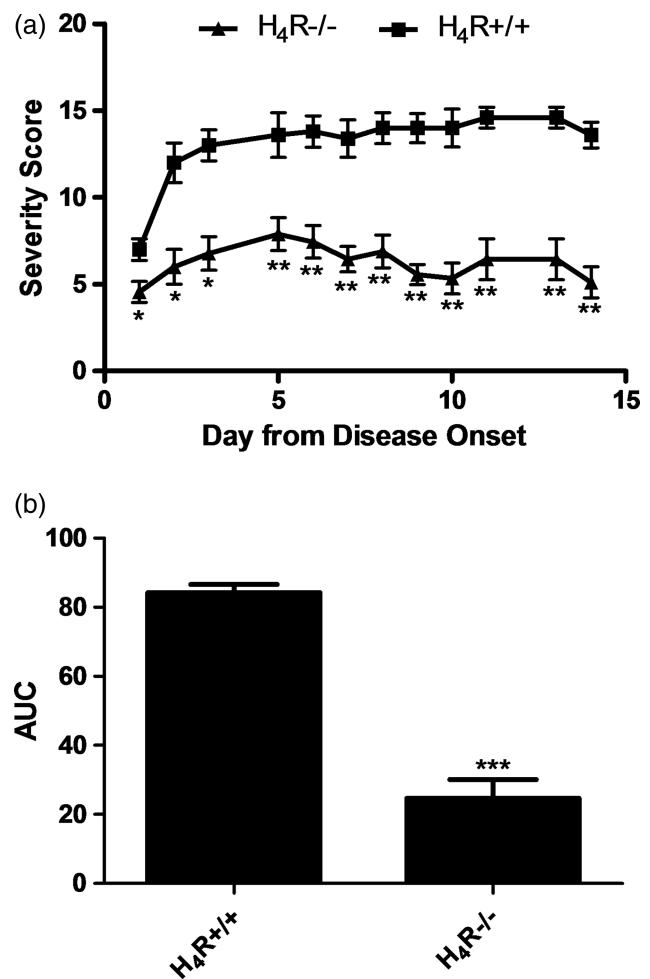


Figure 1 Comparison of histamine H₄ receptor (H₄R)-deficient and wild-type mice in the collagen antibody-induced arthritis model. (A) H₄R-deficient (\blacktriangle , $n=9$) and wild-type (\blacksquare , $n=5$) BALB/c mice were given collagen antibody cocktail and then challenged by intraperitoneal injection of lipopolysaccharide 2 days later. Starting the next day, 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 in (B). A significant reduction in mean disease severity score in H₄R-deficient (H₄R^{-/-}) compared with wild-type (H₄R^{+/+}) mice can be seen when comparing the area under the curve (AUC, B). Statistical significance was calculated for each time point of the severity score or comparing the AUC using a Mann–Whitney test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

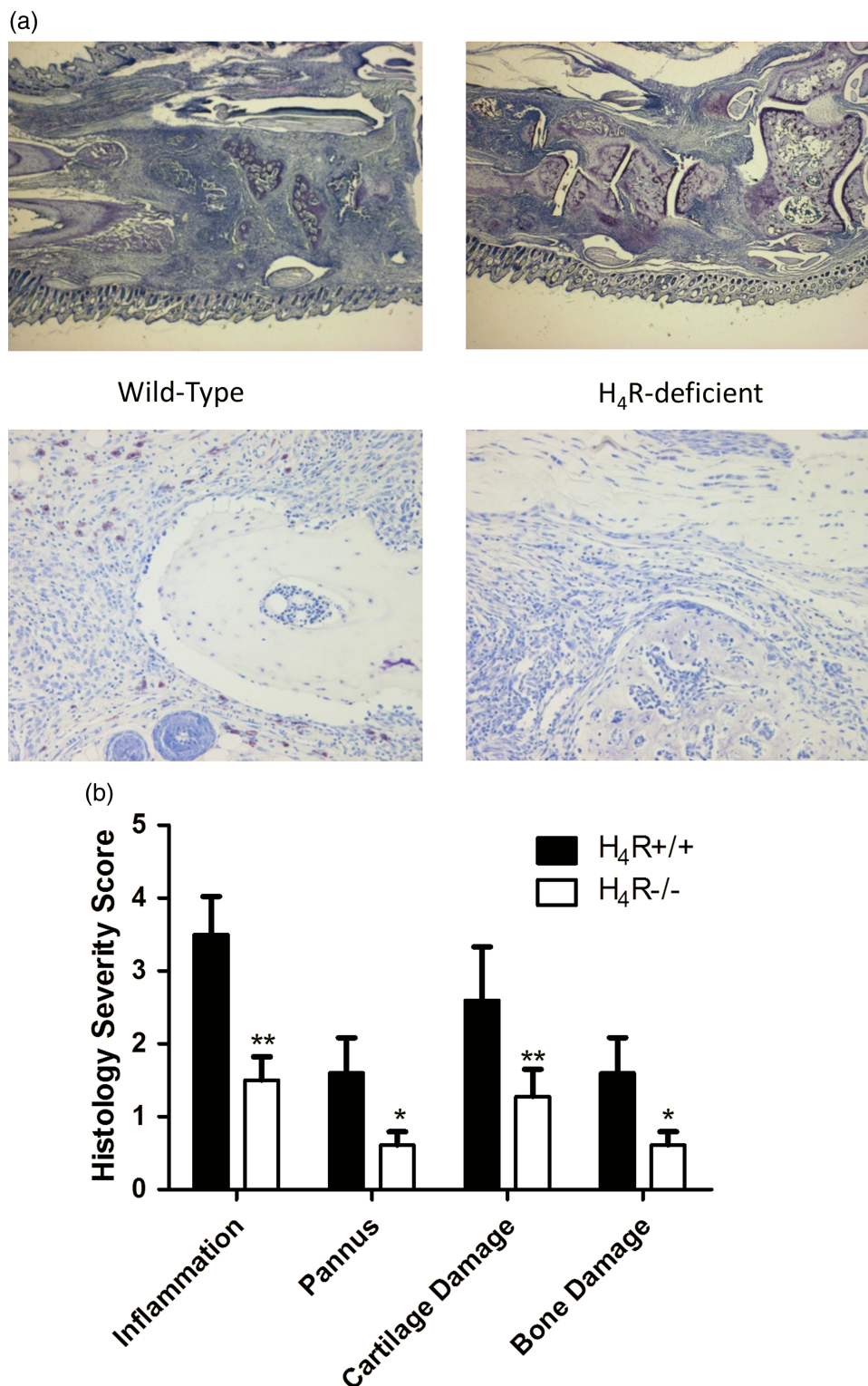


Figure 2 Effects on histology in the collagen antibody-induced arthritis model. (A) Paws were collected for histology and stained with toluidine blue. Representative images are shown for wild-type mice on the left and histamine H₄ receptor (H₄R)-deficient mice on the right. The top images are a 16× magnification, 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.

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.

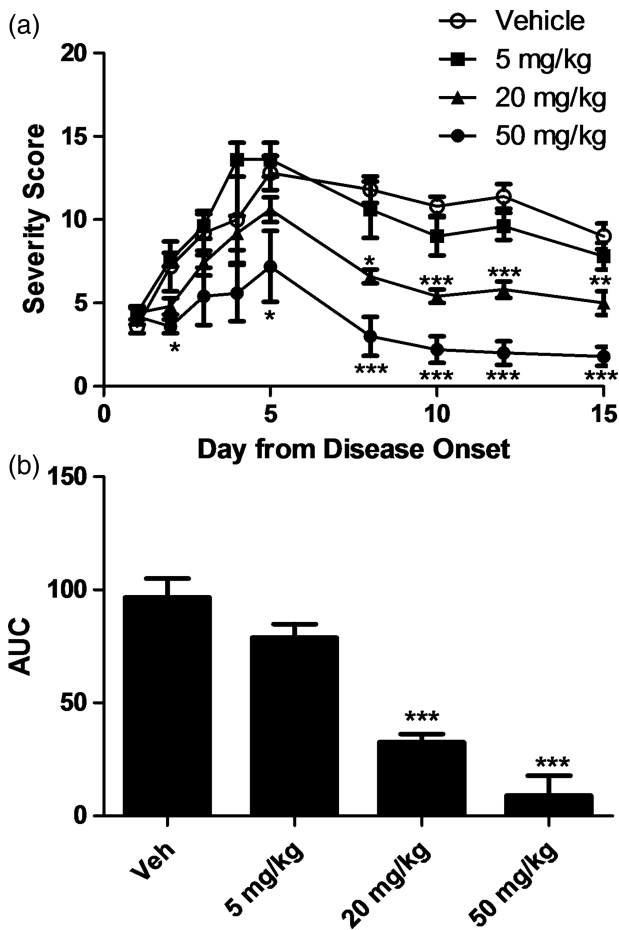


Figure 3 Effects of JNJ 28307474 in the collagen antibody-induced arthritis model. (A) Wild-type BALB/c mice ($n=5$ per group) were given collagen antibody cocktail and then challenged with lipopolysaccharide (LPS) by intraperitoneal injection 2 days later. The day after LPS administration, mice were 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 H_4R 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 H_4R antagonist suggests that the receptor mediates inflammation in this model. To confirm this, studies were carried out in H_4R -deficient mice. However, the standard model is conducted in DBA1/J mice and the H_4R -deficient mice were on the C57BL/6 background, and

therefore the model was adapted to this strain.¹⁵ Before receiving the LPS boost, the incidence of arthritis in the H_4R -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 H_4R -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 H_4R -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 H_4R -deficient mice. Overall, there was a statistically significant decrease in the incidence of arthritis in the H_4R -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 H_4R can mediate inflammation in the mouse CIA model.

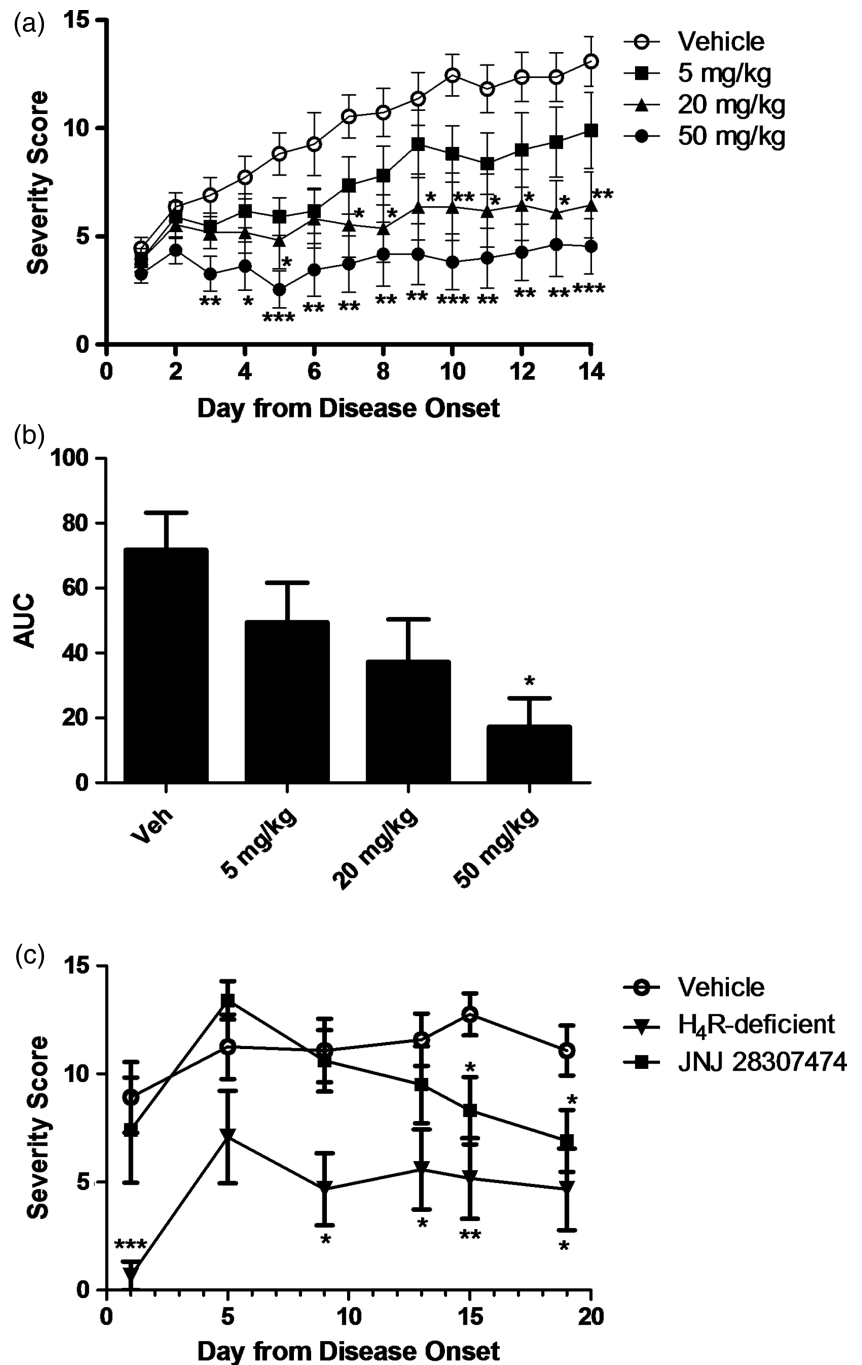
Histological examination was also conducted in this model to illustrate the joint pathology in diseased mice, and representative data are shown in figure 5A. Scoring of inflammation, pannus, cartilage damage and bone damage showed inhibition by 50 mg/kg JNJ 28307474 for all of these variables (figure 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-1 β , monocyte chemoattractant 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 H_4R antagonist on IFN γ was seen, although it was increased in diseased animals (figure 6B).

It is difficult to determine whether the reduction in Th17 cells seen with H_4R antagonist treatment in CIA is due to a direct role of the H_4R 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 H_4R 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 H_4R in Th17 cell development in vivo was confirmed using H_4R -deficient mice. Transferring

Figure 4 Effects of the histamine H₄ receptor (H₄R) in the collagen-induced arthritis model. (A) Wild-type DBA1/J mice (n=11 per group) were immunised with complete Freund's adjuvant (CFA)/collagen and boosted with lipopolysaccharide (LPS) on day 26. Mice were 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 (A) and (B) 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. (C) Wild-type (n=22) or H₄R-deficient (▼, n=12) C57BL/6 mice were immunised twice with CFA/collagen and boosted with LPS on day 28. 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. On day 5, wild-type mice were treated orally with vehicle (○, n=12) or 50 mg/kg twice daily JNJ 28307474 (■, n=10). Statistical significance between H₄R-deficient and wild-type mice or JNJ 28307474- and vehicle-treated mice was determined using a Mann–Whitney test for each time point. *p<0.05, **p<0.01, ***p<0.001.



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 H₄R on 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 7777120 (figure 6E).

A similar effect on IL-17 production can be seen with human cells. A variety of stimuli were able to induce IL-17 production from human peripheral blood mononuclear cells, with the highest levels produced when a combination of anti-CD3, anti-CD28, IL-23 and IL-1β was used (figure 6F). Treatment in vitro with either JNJ 7777120 or JNJ 28307474 was able to reduce the IL-17 level under all stimulation conditions. These results show that, in humans and mice, the H₄R can directly modulate IL-17 production.

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

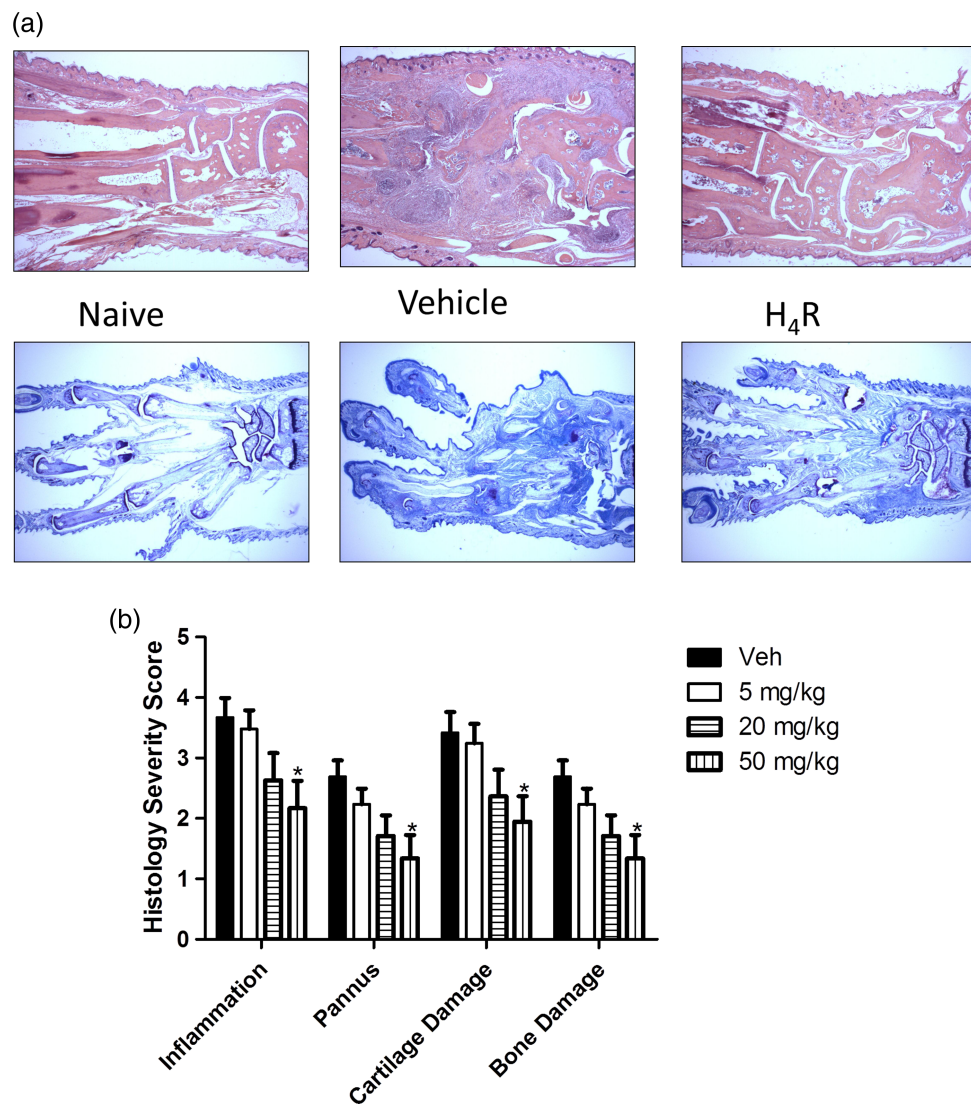
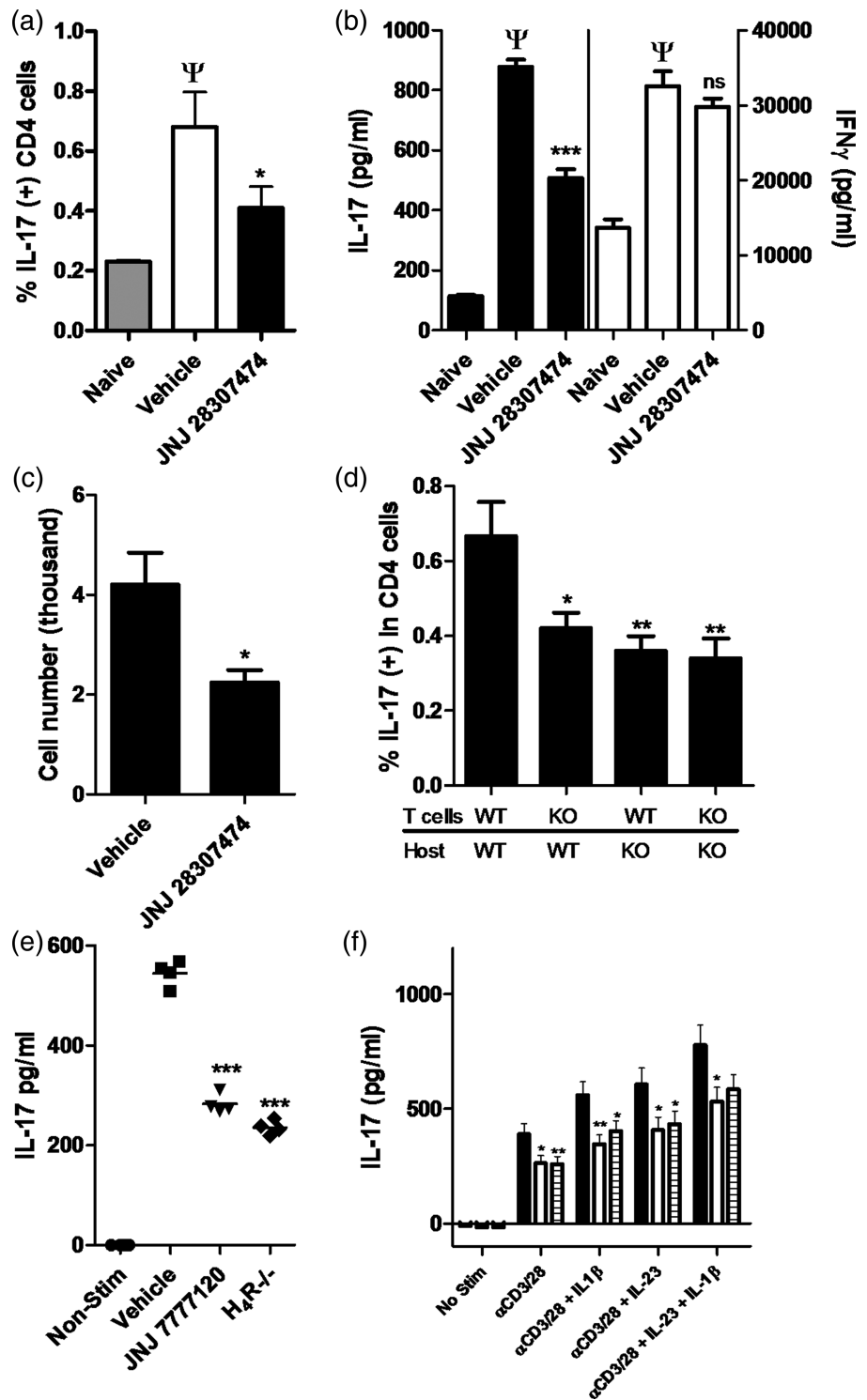


Figure 5 Effects on histology in the collagen-induced arthritis model. (A) Paws were collected for histology and stained with H&E (top) and toluidine blue (bottom). Representative images are shown for naive (unimmunised) mice (left), immunised and vehicle-treated (middle) and immunised and treated with 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.

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.²⁰ 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.²¹ 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.^{7 18 22 23} 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 tended 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 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 777120 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 μ M JNJ 777120 (open bars) or 1 μ M 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. Ψ p<0.01 comparing vehicle with naive 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.



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-deficient 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-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 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.¹⁴ 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,¹⁹ 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.^{27–28} Basophils may also play a role, and it was recently shown that histamine release from basophils amplifies IL-17 release from T cells.²⁹ 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.^{7–30–31} 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,³² 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.^{7–9–18–33} 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.*³⁴). Therefore, H₄R antagonists may provide a safe and effective alternative for the treatment of rheumatoid arthritis.

The work presented here clearly supports a role for the H₄R in arthritis. Importantly, the receptor has effects in both a model of inflammatory arthritis and one of autoimmune arthritis, suggesting that it can affect both innate and adaptive immune responses. There are several potential mechanisms underlying this role including possible effects on Th17 cells. Taken as a whole, the current data suggest that the H₄R can be viewed not necessarily as the initiator of inflammation, but as a potentiator of inflammatory responses. This is evidenced by the partial inhibition of Toll-like receptor (TLR)-mediated cytokine production previously observed^{7–18–22} and the fact that LPS can

cause a flair in severity score in H₄R-deficient mice that then resolves compared with wild-type mice. Therefore, antagonism of the receptor would not be expected to be immunosuppressive, but rather lead to a dampening of the initial inflammatory response, thereby leading to a reduction in inflammation in a variety of disease states, be they allergic or autoimmune in nature.

Acknowledgements The authors acknowledge Alfred Barron for assistance with the statistical analysis, and Lars Karlsson for scientific discussions during the study. Joint histology was performed by Bolder BioPATH (Boulder, Colorado, USA).

Contributors All authors contributed to the conception and design, acquisition of data or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version published.

Competing interests All authors are current or former employees of Janssen Research & Development, L.L.C.

Provenance and peer review Not commissioned; externally peer reviewed.

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