

Immunomodulatory effects of IL-23 and IL-17 in a mouse model of allergic rhinitis

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Summary

Background Interleukin-23 (IL-23) and IL-17 may be involved in the pathogenesis of allergic rhinitis (AR). However, a differentiation of the role of IL-23 and IL-17 has not been performed yet.

Objective The aim of this study was therefore to investigate the immunomodulatory effects of IL-23 and IL-17, using a mouse model of AR.

Methods Anti-IL-23p19 and anti-IL-17 Abs were administrated intranasally during rechallenge in ovalbumin (OVA)-induced AR in BALB/c mice. Immunomodulatory effects were evaluated by measuring nasal rubbing and sneezing occurrences, serum OVA-specific antibodies, Th2 responses (i.e. expression of IL-4, IL-5, IL-13 and IFN- γ genes in nasal mucosa, IL-4⁺ CD4⁺ T cells percentages in superficial cervical lymph nodes (LNs) and IL-4 production in LNs stimulated with OVA *in vitro*), and neutrophil, eosinophil and mast cell recruitment into the nasal mucosa.

Results The effect of IL-17 antagonism was limited to attenuating the Th2 responses and neutrophil and eosinophil infiltration. In contrast, treatment with anti-IL-23p19 Abs markedly reduced nasal rubbing and sneezing events, Th2 responses, serum OVA-specific IgE and IgG1 levels, as well as mucosal neutrophil, eosinophil and mast cell infiltration.

Conclusion and Clinical Relevance IL-17 and IL-23 may play a pathogenic role in an established AR mouse model; with a more pronounced contribution of IL-23 than IL-17.

Keywords allergic rhinitis, immunomodulation, interleukin-17, interleukin-23, mouse model of AR

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Introduction

Interleukin-17 (IL-17; also known as IL-17A) is a member of IL-17 cytokine family produced predominantly by a subpopulation of CD4⁺ T cells, Th17 cells [1], whereas IL-23, a heterodimeric cytokine consisting of a p19 subunit specific for IL-23 and a p40 subunit shared with IL-12, has been shown to play a significant role in the maintenance [2] and acquisition of pathogenic function of Th17 cells [3]. Indeed, IL-23-IL-17 link has been found to be associated with the development of various inflammatory diseases, such as psoriasis [4], arthritis [5] and inflammatory bowel disease (IBD) [6]. However, the role of this IL-23-Th17 cell link in the regulation of allergic airway inflammation remains largely unknown.

To date, the contribution of IL-23 and IL-17 to asthma is controversial; with different studies proposing either a pathogenic [7–9] or a protective role [10]. Studies in patients with allergic rhinitis (AR) indicate that IL-17 is also likely to be involved in the pathogenesis of this condition [11–15]. Indeed, a recent study in IL-17-deficient mice demonstrated that there was a significant decrease in allergic symptoms, serum IgE, eosinophil infiltration and histamine and cysteinyl leukotriene release, thus supporting the pathogenic role of IL-17 in AR [16]. However, the immunoregulatory effects of IL-17 and Th17-regulating cytokine IL-23 in AR are still not clearly understood.

The aim of this study was therefore to investigate the immunomodulatory effects of IL-23 and IL-17 in a

mouse model of AR, through the administration of anti-IL-23p19 and anti-IL-17 Abs.

Methods

Mice

Male BALB/c mice (6- to 8-week-old) were purchased from Experimental Animal Centre (Beijing, China). The animals were housed in horizontal laminar flow cabinets and provided sterile food and water in a specific pathogen-free facility. All experimental procedures were performed under approval from the Animal Care and Use Committee of Capital Medical University.

Experimental protocol

BALB/c mice were divided into six treatment groups of six mice each as follows: (1) control group mice sensitized and challenged with saline; (2) ovalbumin (OVA) group mice sensitized and challenged with OVA; (3) isotype1 group mice treated with isotype Abs for anti-IL-23p19; (4) anti-p19 group mice treated with anti-IL-23p19 Ab; (5) isotype2 group mice treated with isotype Abs for anti-IL-17; (6) anti-IL-17 group mice treated with anti-IL-17 Ab.

The OVA group mice were sensitized on days 1 and 7 by intraperitoneal (i.p.) injection with 50 µg OVA (grade V; Sigma, St Louis, MO, USA) emulsified in 5 mg Al(OH)₃. After a week, the animals were challenged by nasal instillation of 50 µg OVA in 20 µL 0.9% saline per mouse, using a micropipette, on three successive days a week for three consecutive weeks (between day 14 to day 35). After a further 2 weeks, the animals were re-challenged with intranasal OVA for 7 days (from day 49 to day 55). The isotype1, anti-p19, isotype2 and anti-IL-17 group mice were, respectively, given intranasal instillation of goat IgG (isotype anti-

body for anti-IL-23p19), anti-IL-23p19 Ab, rat IgG2a (isotype antibody for anti-IL-17) and anti-IL-17 Ab thirty minutes prior to OVA re-challenge, 10 µg in 20 µL saline per mouse. All antibodies were purchased from R&D Systems Inc. (Minneapolis, MN, USA). Control group mice were sensitized and challenged with saline instead of OVA at all stages.

Two hours after the last challenge on day 55, the animals were killed and the nasal mucosa, blood and superficial cervical lymph nodes (LNs) [17], which drain the nasal mucosa, were harvested from each animal for further analyses. One half of the nasal mucosa samples were used for RNA isolation and the other half were fixed overnight for histocytochemical analysis. Serum was analysed for antigen-specific-antibody. Superficial cervical LNs were analysed for the number of IL-4⁺ CD4⁺ T cells and IL-4 secretion after OVA stimulation. The experimental protocol was as shown in Fig. 1.

Measurement of nasal symptoms

Nasal symptoms were evaluated by counting the number of nasal rubbing occurrences and sneezes over a period of 20 min immediately after the last challenge, by three observers blinded to treatment. The average values for measurements made by the three observers were used for statistical analysis.

Measurement of serum OVA-specific IgE and IgG subclass

Serum OVA-specific IgE, IgG1, and IgG2a were determined by enzyme-linked immunosorbent assay (ELISA). Briefly, ninety-six-well microtiter plates were coated with OVA (10 µg/mL) in 0.05 M carbonate/bicarbonate buffer, pH 9.6 and incubated at 4°C overnight. The wells were blocked by incubation with 4% BSA in PBS for 1 h at room temperature, and following the addition

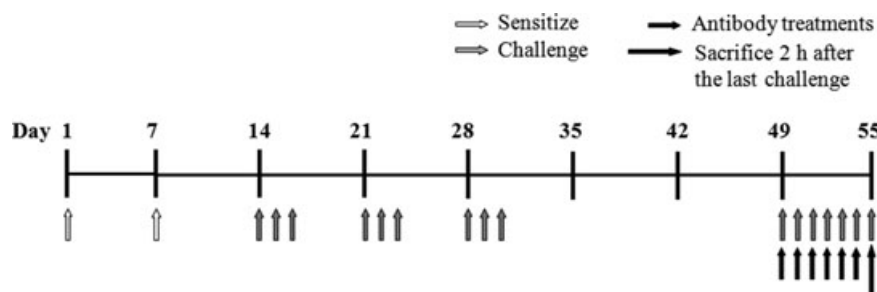


Fig. 1. The experimental protocol. The ovalbumin (OVA) mice were sensitized on days 1 and 7 by i.p. injection of 50 µg OVA plus 5 mg Al(OH)₃. One week later, mice were challenged with 50 µg OVA on three successive days in a week for three consecutive weeks (between day 14 to day 35). Two weeks later, mice were re-challenged seven times (from day 49 to day 55). The isotype1, anti-p19, isotype2 and anti-IL-17 group mice, respectively, were treated by intranasal instillation of goat IgG (isotype antibody for anti-IL-23p19), anti-IL-23p19 Ab, rat IgG2a (isotype antibody for anti-IL-17), and anti-IL-17 Ab 30 min before OVA re-challenge from day 49 to day 55. The normal control mice were sensitized and challenged with saline.

of serum samples to each well, the wells were incubated further for 2 h at room temperature. At the end of incubation, the wells were washed with 0.05% Tween 20 in PBS (PBS-T), and on addition of horseradish peroxidase-conjugated anti-mouse IgE (1 : 4000), IgG1 (1 : 5000) or IgG2a (1 : 5000) (Southern Biotech, Birmingham, AL, USA) to each well, the samples were incubated further for 2 h at room temperature. To avoid cross-reactivity, IgE antibody was pre-cross adsorbed with mouse IgG1, IgG2a, IgG3, IgM and IgA; IgG1 antibody pre-cross adsorbed with mouse IgM, IgG2a, IgG2b, IgG3 and IgA; and IgG2a antibody pre-cross adsorbed with mouse IgM, IgG1, IgG2b, IgG3 and IgA (Southern Biotech). At the end of this incubation, the wells were washed with PBS-T and developed with tetramethylbenzidine. Absorbance (450 nm) was measured with an ELISA plate reader. A standard curve was prepared from serial dilutions of an arbitrary standard and levels of OVA-specific antibodies were expressed as arbitrary unit (AU). To avoid assay saturation, optimal sample dilutions were determined as 1/100 000 for IgG1 and 1/2000 for IgG2a.

Measurement of cytokines

The levels of IL-17 in serum and IL-4 in supernatants of stimulated LN cells were measured by the Cytometric Bead Array (CBA) Flex Set (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. The levels of IL-23 in serum were detected by ELISA (R&D Systems). The limits of detection were 20.5 fg/mL for IL-17 (enhanced sensitivity), and 0.3 pg/mL for IL-4, and 2.28 pg/mL for IL-23.

Real-time polymerase chain reaction

Nasal mucosa was dissected 2 h after the last OVA challenge and immediately frozen in liquid nitrogen for storage at -80°C . Total RNA was isolated with trizol reagent and reverse transcribed into first-strand cDNA using oligo (dT) primer. Real-time polymerase chain reaction (PCR) was performed in the Rotor-GeneTM 6200 HRM (Corbett Life Science, Sydney, Australia).

The expression of IL-17, IL-23p19, Ear1, Ear11 and Fc ϵ r1g mRNA was determined using the Platinum SYBR Green qPCRSuperMixUDG (Invitrogen, Carlsbad, CA, USA). The primer sequences used were as follows: forward primer cggatttgccgtattggg, reverse primer ctgc-tcttggaagatgg for housekeeping GAPDH [18]; forward primer tctctgatgtgtgtgtgct, reverse primer cgtggaacggttgaggtagt for IL-17 [19]; forward primer ccagcagctcttcggaatc, reverse primer tcatatgtcccgctgggtgc for IL-23p19 [20]; forward primer tcttgataaactaattgtctctctcac, reverse primer gcaggatgacaactagctggg for IL-4 [21]; forward primer agcacagtgggtgaaagacatt, reverse primer tccaatgcatactgtgtgattt for IL-5 [21]; forward primer cag-

cttccctggttctctcac, reverse primer ggaatccagggtacacaga for IL-13 [18]; forward primer gcgtcattgaatcacactg, reverse primer tgagctcattgaatgcttg for IFN- γ [18]; forward primer aatgctgttggtgtgtgtggaaat, reverse primer cactggatacatgggactgtcctg for Ear1 [22]; QuantiTect Primer Assay QT00266959 (Qiagen, Hilden, GmbH, Germany) for Ear11; and QuantiTect Primer Assay QT00115717 (Qiagen) for Fc ϵ r1g.

The expression of Mcpt1 and Mcpt2 mRNA was performed using Universal ProbeLibrary probes (Roche Diagnostics Corp., Indianapolis, IN, USA). The primers and probes used were as follows: forward primer ggatgcagaaggagattactgc, reverse primer ccaccgatccacacagagta, probe #63 for housekeeping Beta-actin; forward primer aagaggctgtaaaatgtataagca, reverse primer agaagggtc-ccccagagtc, probe #85 for Mcpt1; and forward primer cctttcgggtttctatgacatc, reverse primer cacatcttccaggttgat, probe #6 for Mcpt2.

Comparative Ct method (delta delta Ct) method was used for relative gene expression analysis.

Draining lymph node cell analysis

Single-cell suspensions of superficial cervical LNs were prepared by passing them through a 100- μm mesh and cultured in RPMI-1640 with 10% FCS at a concentration of 5×10^6 cells/mL.

For detection of IL-4⁺ CD4⁺ T cells (Th2 lymphocyte subsets), single-cell suspensions of LNs were stimulated with 10 ng/mL PMA (Sigma-Aldrich, St Louis, MO, USA), 500 ng/mL ionomycin (Sigma-Aldrich) and 1 μL /mL BD GolgistoTM for 5 h. The stimulated cells were washed twice with staining buffer and stained extracellularly with PerCP-CyTM5.5-conjugated anti-mouse-CD4. Intracellular staining for IL-4 was performed using BD Cytotfix/Cytoperm Kit. After fixation and permeabilization, the cells were stained intracellularly with Alexa Fluor 647-conjugated anti-mouse-IL-4. Isotype-matched antibodies conjugated with PerCP-CyTM5.5 and Alexa Fluor 647 were used as negative controls. All antibodies were purchased from BD Pharmingen. Samples were assessed with a FACSCalibur flow cytometer and data were analysed using the CellQuest software (BD Biosciences).

For measurement of IL-4 cytokine production, single-cell suspensions of LNs were cultured with OVA (200 μg /mL) in 48-well plates in duplicate for 48 h and at the end of incubation, the supernatants were collected for analysis of IL-4 as described above.

Histological analysis of nasal mucosa

Heads of mice were excised and fixed in 10% neutral buffered formalin for 24 h at room temperature. After fixation, the heads were decalcified in 8.8% formic acid

for 6 days and then embedded in paraffin. Samples were cut into 4 µm cross sections and sections were stained with either May-Grünwald-Giemsa for assessment of eosinophil infiltration, with toluidine blue for assessment of mast cell infiltration, or stained by immunohistochemistry for assessment of IL-17, IL-23 and neutrophils.

For IL-17 and IL-23 staining, rabbit anti-IL-17 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-IL23 (abcam, 115759) were used as primary antibodies, and horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (Zhongshanjinqiao, Beijing, China) was used as secondary antibody. For neutrophils staining, rat neutrophil antibody NIMP-R14 (abcam, 2557) was used as primary antibody, HRP-conjugated anti-rat IgG (mouse adsorbed) (abcam, 7097) was used as secondary antibody. As a negative control, the primary antibodies were substituted by isotype- and concentration-matched control antibodies. The positive cells were visualized by further incubation with 3, 3'-diaminobenzidine which rendered the positive cells brown.

Eosinophils, mast cells and neutrophils numbers in the nasal mucosa in five randomly selected tissue sections from each animal were counted using microscopy at $\times 400$ magnification, by two observers who were blinded to the treatment. The mean values of counts made by the two observers were used for statistical analysis.

Statistical analysis

Data were shown as the mean $\pm$ standard deviation (SD). Mann-Whitney *U*-test was used for multiple comparisons among the different groups if the results of the Kruskal-Wallis test showed significant difference. Correlations were tested by the Pearson's correlation. Values of $P < 0.05$ were considered significant. SPSS software for Windows (SPSS Inc., Chicago, IL, USA) was used for data analysis.

Results

Expression of IL-17 and IL-23 in the AR mouse model

Two hours after the last challenge on day 55, the concentration of serum IL-17 and IL-23 was increased in OVA mice compared to control mice (Fig. 2a). Similarly, the IL-17 and IL-23 mRNA (Fig. 2b) and protein (Fig. 2c) expression in the nasal mucosa was increased in OVA mice.

Effect of p19 and IL-17 blockade on nasal symptoms in the AR mouse model

The number of nasal rubbings and sneezes was increased significantly in OVA and isotypes1 and 2 mice compared

to control mice (Fig. 3). Blockade of p19 significantly reduced the nasal rubbing and sneezes, whereas no decrease was observed after IL-17 blockade (Fig. 3). Moreover, the number of eosinophils and mast cells were correlated with the nasal rubbing ($r = 0.54$, $P < 0.01$ for eosinophils; $r = 0.58$, $P < 0.01$ for mast cells).

Inhibition of Th2 responses by p19 and IL-17 blockade

Gene expression of Th2 cytokines (IL-4, IL-5 and IL-13) in nasal mucosa was up-regulated in OVA and isotypes1 and 2 groups and the up-regulation of these genes was inhibited by p19 and IL-17 blockade (Fig. 4a). However, gene expression of Th1 cytokine (IFN- γ) in nasal mucosa was not significantly different among the groups (Fig. 4a).

Similarly, blockade of p19 and IL-17 inhibited IL-4⁺ CD4⁺ T cells (Figs. 4b and c) and IL-4 levels (Fig. 4d) in superficial cervical LNs in OVA and isotypes1 and 2 mice.

Effect of p19 and IL-17 blockade on serum OVA-specific antibodies

The OVA-specific IgE, IgG1 and IgG2a levels were significantly increased in OVA and isotypes1 and 2 mice compared to control animals. Blockade of p19 significantly reduced the levels of OVA-specific IgE and IgG1. In contrast, blockade of IL-17 had no effect on OVA-specific IgE, IgG1 or IgG2a (Fig. 5).

Inhibition of neutrophil infiltration by anti-p19 and anti-IL-17 Abs

The numbers of neutrophils were significantly increased in nasal mucosa of OVA and isotypes1 and 2 mice compared with control mice; and significantly attenuated by treatment with anti-p19 and anti-IL-17 Abs (Fig. 6).

Inhibition of eosinophil infiltration by anti-p19 and anti-IL-17 Abs

The numbers of eosinophils were significantly increased in nasal mucosa of OVA and isotypes1 and 2 mice compared with control mice; and this increase was significantly attenuated by blockade with anti-p19 and anti-IL-17 Abs (Figs. 7a and b).

Similarly, expression of eosinophil-associated ribonuclease (Ear)1 and Ear11 genes was significantly increased in OVA and isotypes1 and 2 mice, and inhibited by treated with anti-p19 and anti-IL-17 Abs (Fig. 7c). The relative mRNA expression of Ear1 and Ear11 was significantly correlated with the number of eosinophils in the nasal mucosal ($r = 0.74$, $P < 0.001$ for Ear1; $r = 0.69$, $P < 0.001$ for Ear11).

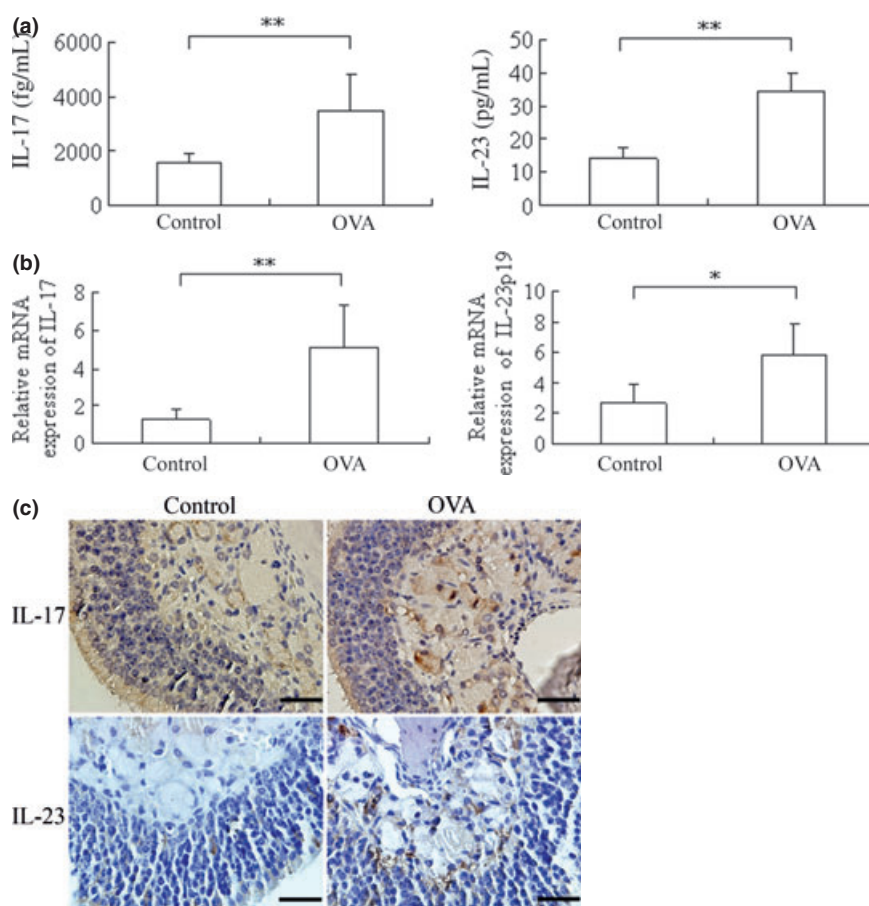


Fig. 2. Up-regulation of IL-17 and IL-23 in the mouse model of AR. Blood and nasal mucosa were collected 2 h after the last challenge at day 55 and analysed for serum IL-17 and IL-23 levels (a), relative mRNA expression of IL-17 and IL-23 (b), and expression of IL-17 and IL-23 protein (c) in nasal mucosa. The control group mice were sensitized and challenged with saline, and the OVA group mice were sensitized and challenged with OVA. Data are expressed as the mean $\pm$ SD ($n = 6$ in each group). Results are representative of two independent experiments with similar results. * $P < 0.05$, ** $P < 0.01$. Scale bars, 20 μ m.

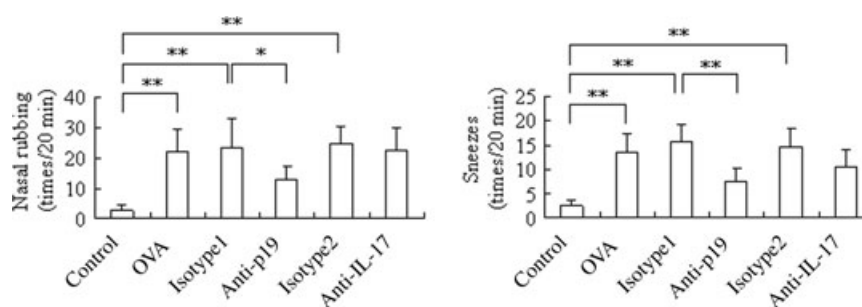


Fig. 3. Effect of p19 and IL-17 blockade on nasal rubbings and sneezes. Control: sensitized and challenged with saline; ovalbumin (OVA): sensitized and challenged with OVA; isotype1: treated with control Abs for anti-p19; anti-p19: treated with anti-p19; isotype2: treated with control Abs for anti-IL-17; anti-IL-17: treated with anti-IL-17. The number of nasal rubbings and sneezes was counted for 20 min immediately after the last challenge. Data are expressed as the mean $\pm$ SD ($n = 6$ in each group). Results are representative of two independent experiments with similar results. * $P < 0.05$, ** $P < 0.01$.

Effect of p19 and IL-17 blockade on mast cell infiltration

The numbers of mast cells in nasal mucosa were significantly increased in OVA and isotypes1 and 2 mice, and

significantly decreased by anti-p19 Abs treatment, but not by anti-IL-17 Abs treatment (Figs. 8a and b).

Consistent with mast cell recruitment in nasal mucosa, expression of Mcpt1 and Mcpt2 genes was significantly increased in OVA and isotypes1 and 2 mice

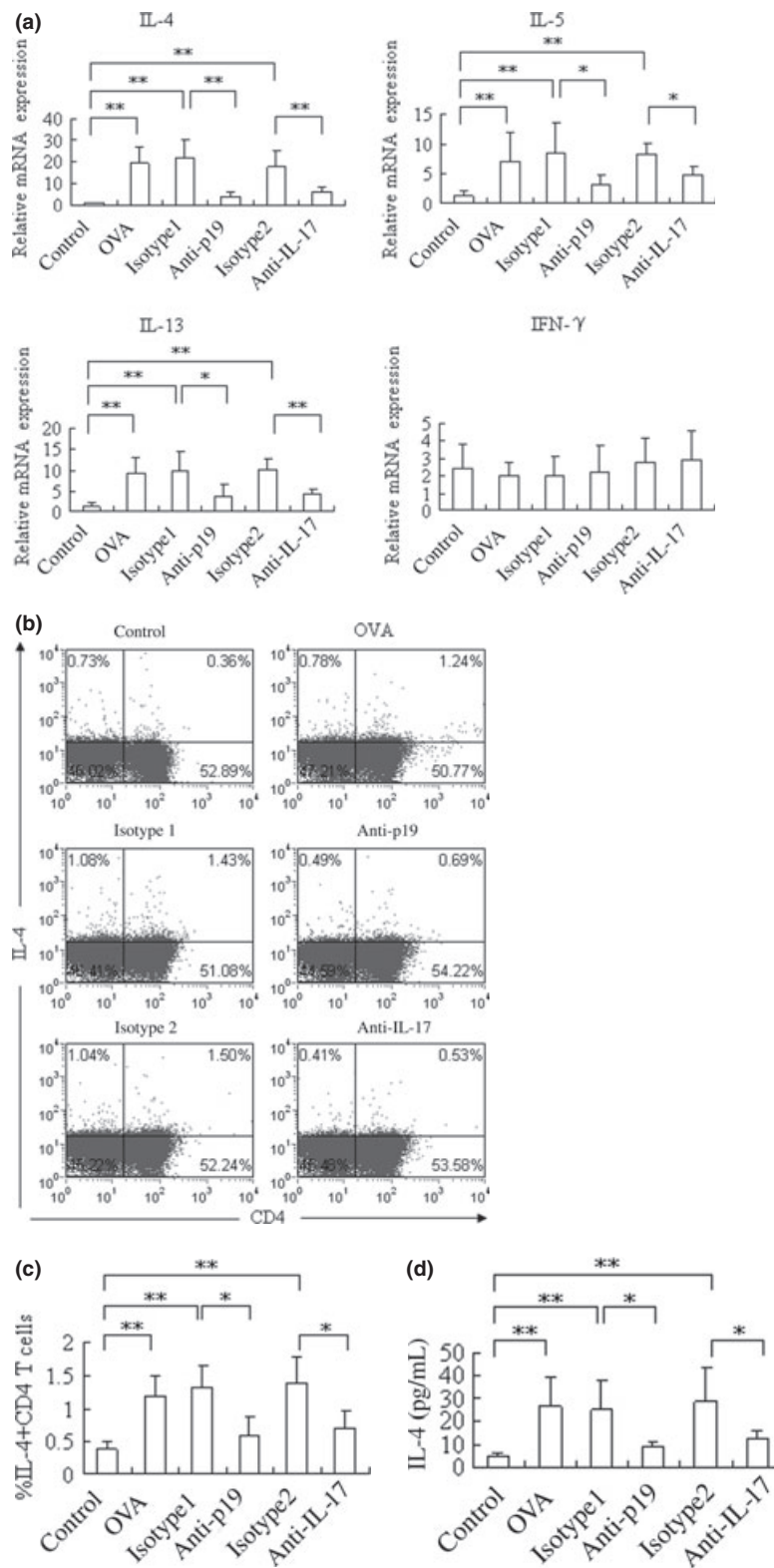


Fig. 4. Inhibition of Th2 responses by p19 and IL-17 blockade. (a) Expression of IL-4, IL-5, IL-13 and IFN- γ genes in nasal mucosa; (b–c) Percentages of IL-4⁺ CD4⁺ T cells in LNs, (b) Representative flow cytometry dot plots for each treatment group. All plots were gated on the lymphocyte population based on forward and side scatter. Numbers in black in the upper right quadrant indicate the percentage of IL-4⁺ CD4⁺ T cells among lymphocyte population, (c) Percentages of IL-4⁺ CD4⁺ T cells for each treatment group; (d) IL-4 secretion by LN cells stimulated with OVA *in vitro*. Data are expressed as the mean $\pm$ SD ($n = 6$ in each group). Results are representative of two independent experiments with similar results. * $P < 0.05$, ** $P < 0.01$.

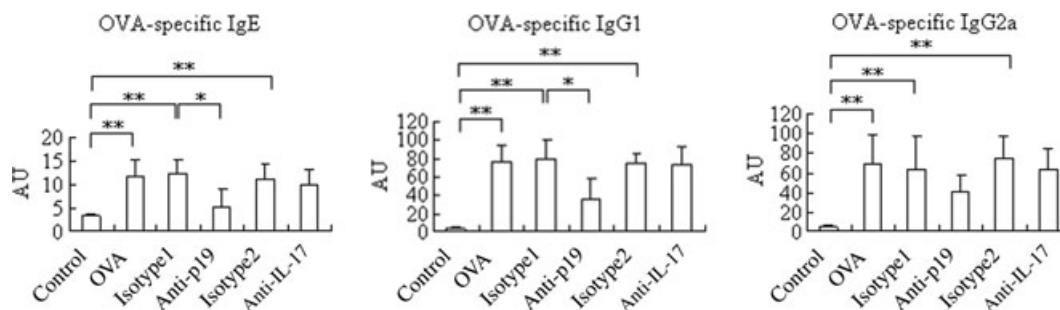


Fig. 5. Estimation of serum antigen-specific-antibody responses. Serum was collected two hours after the last challenge, and the OVA-specific IgE, IgG1 and IgG2a levels were analysed by ELISA. Levels of OVA-specific antibodies were expressed as arbitrary unit (AU). Data are expressed as the mean $\pm$ SD ($n = 6$ in each group). Results are representative of two independent experiments with similar results. * $P < 0.05$, ** $P < 0.01$.

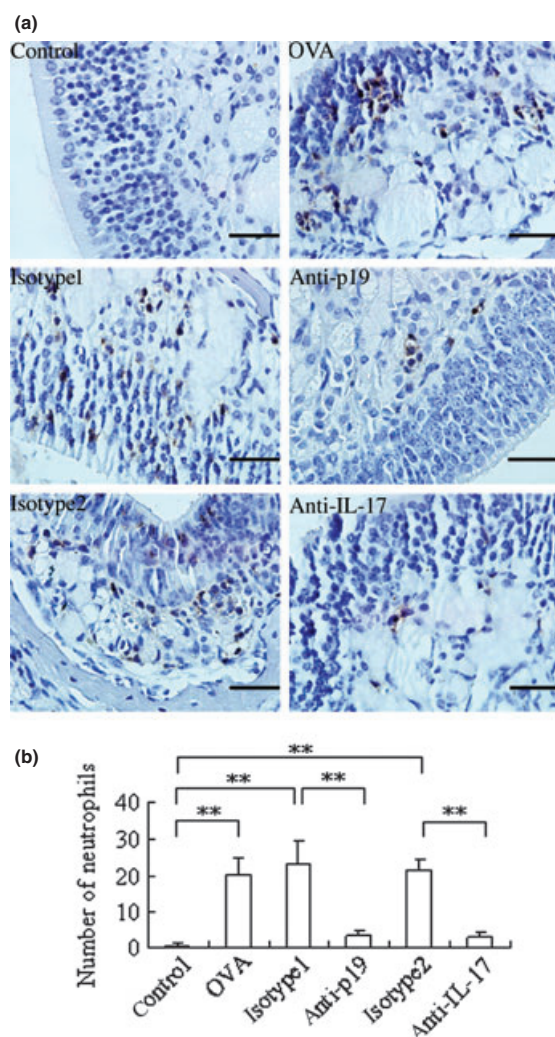


Fig. 6. Effect of p19 and IL-17 blockade on neutrophil infiltration in nasal mucosa. (a) Representative images of neutrophil infiltration of each group. Positive neutrophil cells are stained brown. Scale bars, 20 μ m; (b) Quantitative analysis of neutrophils. Data are expressed as mean $\pm$ SD of each field (at a magnification of $\times 400$) in individual groups of mice. Six fields from five sections of individual mice were counted. Results are representative of two independent experiments with similar results. * $P < 0.05$, ** $P < 0.01$.

compared to control mice. Blockade of p19 significantly reduced the expression of Mcpt1 and Mcpt2 genes, whereas IL-17 blockade had no effect on the expression of these genes (Fig. 8c).

In this study, the increased expression of Fc ϵ rlg gene in OVA and isotypes1 and 2 mice was down-regulated by p19 blockade, but not by IL-17 blockade (Fig. 8c).

Discussion

Previous studies have shown the involvement of IL-17 and IL-23 in patients with AR; however, the potential pathogenic mechanisms associated with these mediators are largely unknown. The goal of this study was therefore to explore the immunoregulatory effect of IL-17 and IL-23 in an OVA-induced AR mouse model.

Most of the studies investigating allergic airway disease have been performed in models of primary allergen challenge which is different from the actual human exposure history. To mimic the natural development of AR more closely, the mice in this study were re-challenged two weeks after primary challenge, and the blocking antibodies were administered during re-challenge; with the aim to investigate the role of IL-17 and IL-23 in already established allergic airway disease. Moreover, neutralizing antibodies for IL-17 and IL-23 were administered locally in this study, to avoid systemic adverse effects directly.

In our mouse model of AR, we found the up-regulation of IL-17 and IL-23 and observed that anti-IL-23p19 Ab and anti-IL-17 Ab treatment had different effects. Anti-IL-23p19 Ab treatment significantly alleviated nasal symptoms, accompanied by reduced Th2 responses, serum OVA-specific IgE, as well as nasal neutrophil, eosinophil and mast cell infiltration. However, anti-IL-17 Ab treatment only inhibited the Th2 responses and nasal neutrophil and eosinophil infiltration. Although IL-23 has been shown to be essential for Th17 response [2, 3], the different effects between anti-IL-23p19 Ab

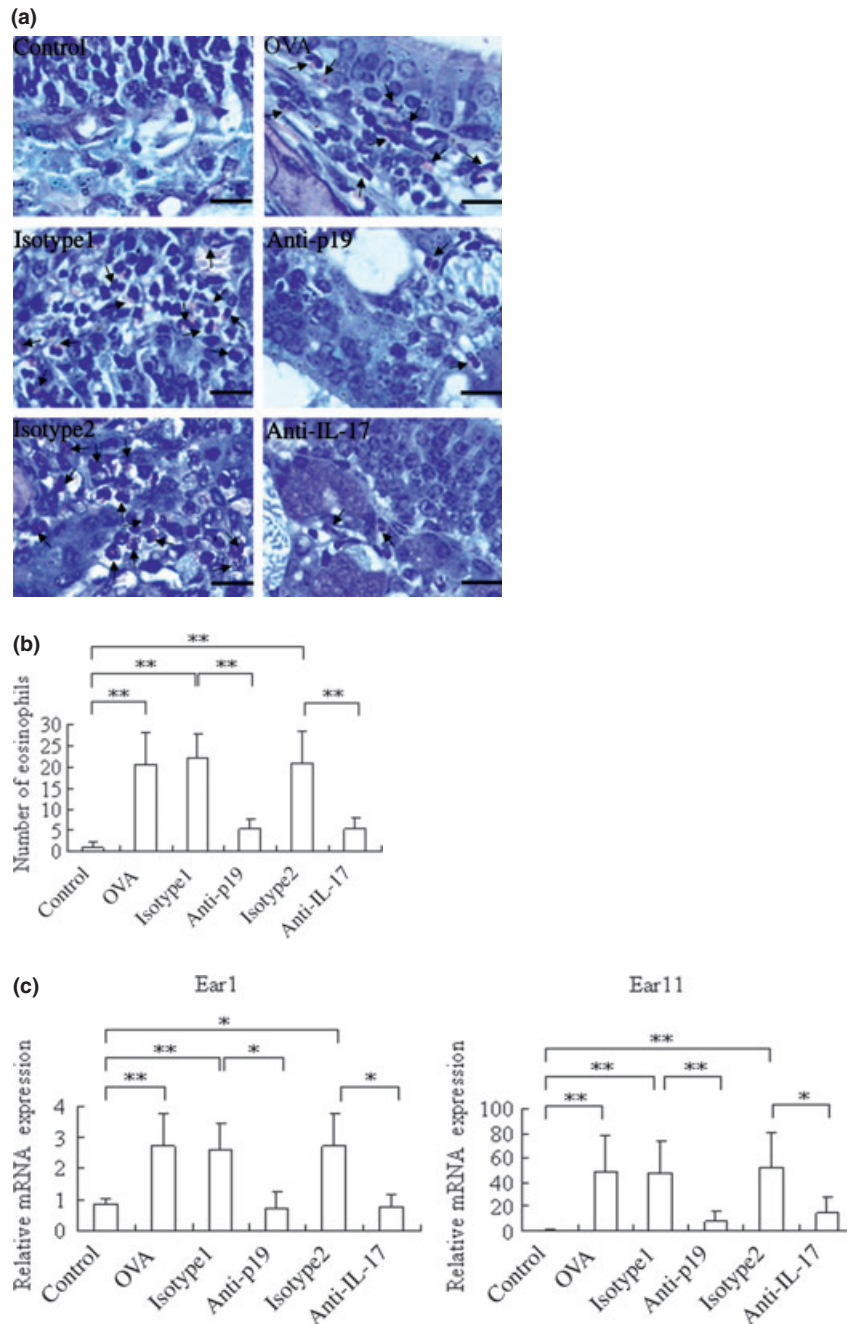


Fig. 7. Effect of p19 and IL-17 blockade on eosinophil infiltration in nasal mucosa. (a) Eosinophil infiltration in nasal mucosa seen by May-Grünwald-Giemsa staining. Representative images of each group are shown. Eosinophils are indicated by arrows. Scale bars, 10 μ m; (b) Quantitative analysis of eosinophils. Data are expressed as mean $\pm$ SD of each field (at a magnification of $\times 400$) in individual groups of mice. Six fields from five sections of individual mice were counted; (c) Expression of eosinophil-related genes, Ear1 and Ear11, in the nasal mucosa. Data are expressed as the mean $\pm$ SD ($n = 6$ in each group). Results are representative of two independent experiments with similar results. * $P < 0.05$, ** $P < 0.01$.

and anti-IL-17 Ab in our study indicate that IL-23 may have functions other than promoting the Th17 effect response in the pathogenesis of AR.

Some previous studies have shown that IL-17 was involved in the recruitment of neutrophils into the airways [23, 24]. In our study, both IL-23p19 and IL-17

blockade inhibited the increased nasal neutrophil infiltration, suggesting that IL-23 may also contribute to neutrophil recruitment into the nasal mucosa, possibly via the induction of IL-17. Our findings for neutrophil recruitment into the nasal mucosa in AR are consistent with a previous study of allergic airway inflammation,

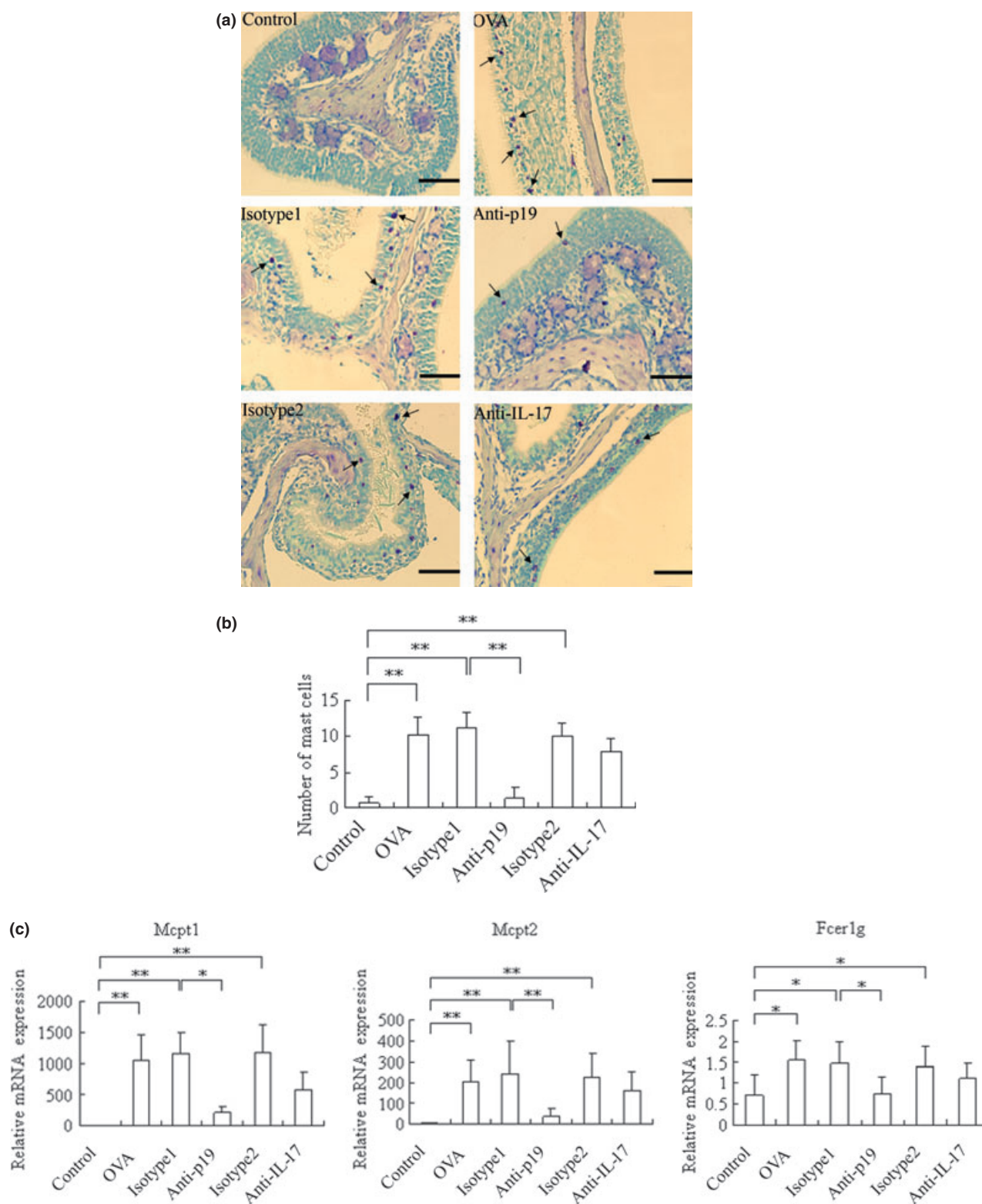


Fig. 8. Effect of p19 and IL-17 blockade on mast cell infiltration in nasal mucosa. (a) Mast cell infiltration in nasal mucosa seen by toluidine blue staining. Representative images of each group are shown. Mast cells are indicated by arrows. Scale bars, 40 μ m; (b) Quantitative analysis of mast cells. Data are expressed as mean $\pm$ SD of each field (at a magnification of $\times 400$) in individual groups of mice. Six fields from 5 sections of individual mice were counted; (c) Expression of mast cell-related genes, Mcpt1, Mcpt2 and Fc ϵ r1g, in the nasal mucosa. Data are expressed as the mean $\pm$ SD ($n = 6$ in each group). Results are representative of two independent experiments with similar results. * $P < 0.05$, ** $P < 0.01$.

which showed the involvement of IL-23 and Th17 in neutrophil infiltration [7].

Eosinophil infiltration is a hallmark of allergic airway inflammation. The role of IL-17 in eosinophil recruitment remains controversial, with different studies proposing either an enhancing [16] or an inhibitory [10, 23] role. Our study found that anti-p19 and anti-IL-17 Abs inhibited both eosinophil numbers and eosinophil-associated genes (Ear1 and Ear11) expression in the nasal mucosa, supporting an enhancing role of these two cytokines in eosinophil recruitment. Eosinophil infiltration is related to Th2 responses, especially IL-5 secreted by Th2 cells [25]. In our study, both anti-p19 and anti-IL-17 Abs inhibited IL-5 gene expression in nasal mucosal, further supporting the pro-inflammatory role of IL-17 and IL-23 in AR. However, IL-23 may regulate Th2 responses either directly or via IL-17 because it has been shown to enhance antigen-induced Th2 cell differentiation in the absence of IL-17 [7, 26].

There is evidence that mast cell recruitment is often accompanied by eosinophil infiltration and that there is an interaction between mast cells and eosinophils [27–29]. While our findings for inhibition of eosinophil infiltration by blockade of both p19 and IL-17 would presume that mast cell infiltration would also be similarly inhibited, our study showed that treatment with p19 Abs, but not for IL-17 Ab, decreased allergen-induced mast cell infiltration in the nasal mucosa. Other studies have demonstrated that mast cell migration and activation is related to IgE levels [30–32]. Our findings are also consistent with these findings for an association between mast cell infiltration and serum OVA-specific IgE levels, and thus support the notion that mast cell infiltration in AR is predominantly related to IgE.

Although the role of IL-23 in allergic airway inflammation has been reported consistently, the role of IL-17 is controversial [10, 16, 23, 33]. The discrepancy in various studies can be explained by different experimental conditions, such as protocol and the route of sensitization and challenge, and the route and the phase of IL-17 treatment (i.e. blocking or enhancing). Our study differs from the other studies mainly in that our anti-IL-17 Ab was administered in re-challenge phase. Our study has indicated that although IL-17 has a patho-

genic role in this well-established mouse model of AR, its effects are limited to the Th2 responses and nasal neutrophil and eosinophil infiltration. The failure of anti-IL-17Ab to inhibit OVA-specific Ig-E and mast cell infiltration in our study may contribute to its failure to alleviate nasal symptoms, because IgE levels are related to mast cell migration and activation [32] and secretion of histamine, which is the main mediator that stimulates nasal rubbing and sneezing [34]. The limited effects of anti-IL-17 Ab in our study indicate that IL-17 may not be a crucial pathogenic mediator in this mouse model of AR. However, further studies with higher doses of anti-IL-17 Ab, and/or different routes of administration (e.g. giving intraperitoneally), as well as investigations in IL-17-deficient mice need to be done to confirm this.

In conclusion, this study demonstrates a pathogenic role of IL-17 and IL-23 in an established mouse model of AR; with a greater contribution of IL-23/p19 than IL-17. Moreover, our present findings raise the possibility that IL-23/p19 blockade may be a novel therapeutic method for AR patients.

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Conflicts of interest

The authors declare no conflicts of interest.

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