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ORIGINAL ARTICLE

Macrolide therapy suppresses key features of experimental steroid-sensitive and steroid-insensitive asthma

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ABSTRACT

Background Steroid-insensitive endotypes of asthma are an important clinical problem and effective therapies are required. They are associated with bacterial infection and non-eosinophilic inflammatory responses in the asthmatic lung. Macrolide therapy is effective in steroid-insensitive endotypes, such as non-eosinophilic asthma. However, whether the effects of macrolides are due to antimicrobial or anti-inflammatory mechanisms is not known.

Objective To determine and assess the efficacy of macrolide (ie, clarithromycin) and non-macrolide (ie, amoxicillin) antibiotic treatments in experimental models of infection-induced, severe, steroid-insensitive neutrophilic allergic airways disease (SSIAAD), compared with steroid-sensitive AAD and to delineate the antimicrobial and anti-inflammatory effects of macrolide therapy.

Methods We developed and used novel mouse models of *Chlamydia* and *Haemophilus* lung infection-induced SSIAAD. We used these models to investigate the effects of clarithromycin and amoxicillin treatment on immune responses and airways hyper-responsiveness (AHR) in Ova-induced, T helper lymphocyte (Th) 2 -associated steroid-sensitive AAD and infection-induced Th1/Th17-associated SSIAAD compared with dexamethasone treatment.

Results Clarithromycin and amoxicillin had similar antimicrobial effects on infection. Amoxicillin did attenuate some features, but did not broadly suppress either form of AAD. It did restore steroid sensitivity in SSIAAD by reducing infection. In contrast, clarithromycin alone widely suppressed inflammation and AHR in both steroid-sensitive AAD and SSIAAD. This occurred through reductions in Th2 responses that drive steroid-sensitive eosinophilic AAD and tumour necrosis factor α and interleukin 17 responses that induce SSIAAD.

Conclusions Macrolides have broad anti-inflammatory effects in AAD that are likely independent of their antimicrobial effects. The specific responses that are suppressed are dependent upon the responses that dominate during AAD.

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Key messages

What is the key question?
- Can macrolides be used to treat infection-induced steroid-insensitive severe asthma and how does this work?

What is the bottom line?
- Clarithromycin suppresses severe, steroid-insensitive allergic airways disease through its anti-inflammatory effects on tumour necrosis factor-α/interleukin-17 immune responses that are largely independent of its antimicrobial effects.

Why read on?
- This study demonstrates how macrolide therapies may be effective in infection-induced steroid-insensitive asthma.

INTRODUCTION

Inhaled corticosteroids are the mainstay therapy for the management of asthma. However, they only suppress the symptoms and not the underlying causes of the disease. Significantly, between 5% and 10% of asthmatics are refractory to steroid treatment,[1] and this group accounts for >50% of asthma-associated healthcare costs.[2] Steroid-insensitive asthmatics typically have more severe disease, which is commonly characterised by non-eosinophilic or neutrophilic airway inflammation.[3] Effective therapies are urgently required for steroid-insensitive asthma. However, the limited understanding of the mechanisms and aetiological factors that underpin steroid-insensitivity has restricted the development of such treatments.

Clinical studies show associations between increases in the expression of T helper lymphocyte type (Th)1 and Th17 cytokines with neutrophilic inflammation and severe, steroid-insensitive (SSI) asthma.[2 4] Experimental studies show that adoptive transfer of Th1 and Th17 cells induce a phenotype of allergic airways disease (AAD) in mice that is characterised by increased neutrophilic airway inflammation and is more resistant to steroid treatment than Th2 cell-induced AAD.[5 6] These studies suggest that Th1 and/or Th17 immune responses in the lung may result in more severe endotypes of asthma that are steroid-insensitive.

Numerous clinical studies have linked *Chlamydia pneumoniae* (*Cpn*) and *Haemophilus influenzae* (*Hi*) infections to steroid-insensitive endotypes such

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as neutrophilic asthma. *Cpn* lung infection induces strong neutrophilic inflammation and potent Th1 and Th17 responses, which are required to clear the infection.[7 8] Furthermore, increased airway neutrophil numbers in SSI asthmatics predict the presence of *Chlamydia*.[9] *Hi* infections also induce neutrophilic inflammation and Th17 responses.[10 11] One study showed that in neutrophilic asthmatics, 43% were colonised by bacteria, and *Hi* was most commonly isolated.[12] We previously showed that neutrophilic asthmatics had significantly higher loads of bacteria. *Hi* was detected in 60% of these patients, who were taking higher doses of steroids.[13] Together, these studies provide strong associations between infections that induce Th1/Th17 responses and neutrophilic inflammation with steroid-insensitivity in severe asthma.

Specific treatments for infection-induced SSI asthma are not widely used and alternative approaches are needed. One strategy is the use of infection- and/or immune-targeted therapies such as antibiotics, particularly macrolides. Some antibiotics, such as β-lactams like amoxicillin, have antimicrobial but not broad anti-inflammatory properties. Macrolides are potent antimicrobial agents and first-line treatments for some bacterial infections. They inhibit bacterial protein synthesis, adherence, motility and biofilm formation.[14 15] Macrolides also have immunomodulatory properties, and in severe asthma, clarithromycin suppresses airway inflammatory cell infiltration and airway hyperresponsiveness (AHR) and enhances steroid responses.[16 17] Importantly, however, it is unknown whether the effects of macrolides are underpinned by their antimicrobial or anti-inflammatory properties. Elucidating the effects of macrolides may highlight their potential use and further the understanding of how we may optimally treat SSI asthma.

Here, we developed a novel mouse model of *Chlamydia* infection-induced, severe, steroid-insensitive, neutrophilic AAD (SSIAAD). We used it as well as a model of *Hi*-induced SSIAAD we previously developed[18] to compare the efficacy of non-macrolide and macrolide antibiotics (amoxicillin and clarithromycin) that are in widespread clinical use, in treating this endotype. We show that clarithromycin, but not amoxicillin, is an effective treatment for steroid-sensitive AAD and SSIAAD even in the absence of steroids, and efficacy results from anti-inflammatory rather than antimicrobial properties.

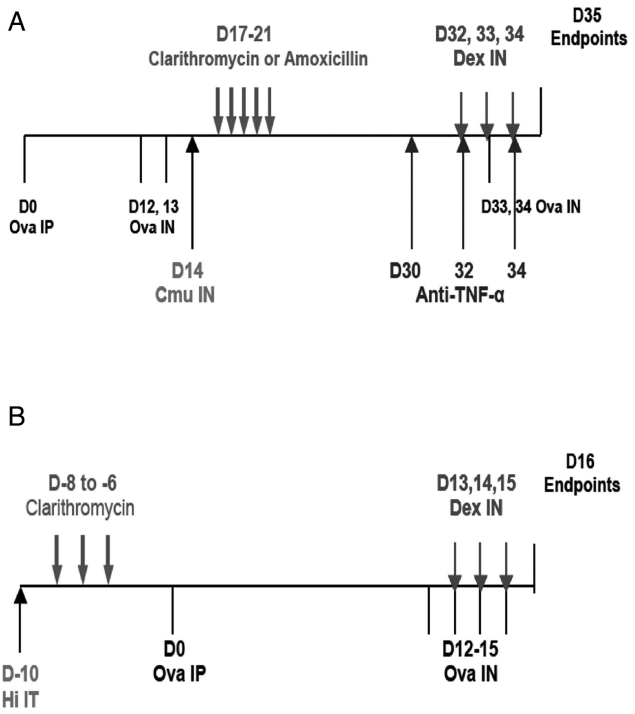

Figure 1 Novel models of infection-induced, severe, steroid-insensitive, neutrophilic allergic airways disease (SSIAAD). Mice were sensitised to ovalbumin (Ova) by intraperitoneal (IP) injection (d0) and AAD was induced by intranasal (IN) Ova challenge (d12–13) followed by rechallenge (d33–34, A). Infected groups were inoculated IN with 100 inclusion-forming units of *Chlamydia muridarum* in between the two sets of Ova challenges (Cmu; d14). Antibiotics, dexamethasone (DEX) and anti-tumour necrosis factor α (anti-TNF-α) treatments were administered by gavage (d17–21) and IN (DEX d32–34 and anti-TNF-α d30, 32, 34), respectively. Uninfected controls were sham-inoculated with sucrose phosphate glutamate, and non-allergic controls were sham-sensitised with saline (Sal). Mice were sensitised to Ova IP (d0) and AAD induced by IN Ova challenge (d12–15, B). Infected groups were inoculated with 5×10^5 colony forming units of *Haemophilus influenzae* (*Hi*) intratracheally (IT; d-10). Antibiotic and DEX treatments were administered d-8 to d-6 and d13–15, respectively. Uninfected controls received phosphate buffered saline, and non-allergic controls were sham-sensitised with Sal. All features of AAD were assessed 24 h after the final Ova challenge.

METHODS

See online supplement for additional details.

Experimental models

In our new model of SSIAAD, female (6–8 weeks old) BALB/c mice were intraperitoneally (IP) sensitised to ovalbumin (Ova, 50 µg (Sigma-Aldrich, Castle Hill, Australia)), with the adjuvant alum (1 mg Rehydrogel (Reheis, Berkeley Heights, New Jersey, USA) in 200 µL 0.9% saline) on day 0.[19] Mice were then intranasally (IN) challenged with Ova on d12–13 and d33–34 (10 µg; 50 µL sterile saline (figure 1A)). On d14, mice were inoculated IN with the natural mouse pathogen *Chlamydia muridarum* (Cmu; 100 inclusion-forming units, ATCCVR-123, 30 µL sucrose phosphate glutamate buffer (SPG)).[19 20] Dexamethasone (DEX) was administered IN (2 mg/kg; 50 µL phosphate buffered saline (PBS)) on d32–34 with Ova challenges (figure 1A). We investigated the broader applicability of our data in another established model using *Hi* infection. Mice were inoculated intratracheally (IT) with 5×10^5 colony-forming units of non-typeable *Hi* (NTHi-289, 30 µL PBS) 10d prior (d-10) to Ova IP sensitisation and challenged on d12–15 (figure 1B). Mice received DEX (1 mg/kg; 50 µL PBS) IN on d13–15.[10] Controls were sham-sensitised with saline, and sham-inoculated with SPG (*Chlamydia*-induced AAD) or PBS (*Hi*-induced AAD). Mice were sacrificed 24 h after the final challenge and features of AAD were assessed.

Antibiotic treatment

Amoxicillin:clavulanate or clarithromycin (5 mg/kg in 200 µL PBS) was administered by oral gavage on d17–21 (*Chlamydia*-induced SSIAAD (figure 1A)) or d-8 to d-6 (*Hi*-induced SSIAAD (figure 1B)). Controls received PBS.

Anti-TNF-α therapy

Anti-tumour necrosis factor α (Anti-TNF- α; 50 µg, 50 µL PBS, (BioXCell, West Lebanon, USA)) monoclonal antibody was administered IN to *Chlamydia*-infected groups with AAD (Ova/Cmu/αTNF-α) on d30, 32 and 34.

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Airway inflammation

Differential leucocyte counts were obtained from May-Grunwald Giemsa stained bronchoalveolar lavage fluid (BALF) cells, using light microscopy.[10]

Lung function

AHR was measured by anaesthetised, cannulated mice using the Scireq flexiVent FX1 system (Montreal, Canada) for all *Chlamydia* experiments, or Buxco Electronic resistance and compliance system (Sharon, Connecticut, USA) for all *Hi* experiments.[10 19] Data are represented as airways resistance at 10 mg/kg methacholine (figures 1–7) and as dose responsive curves (see online supplement).

Lung mRNA expression

RNA was extracted from homogenised whole lung tissue and reverse transcribed. Relative abundance of cytokine complementary DNA was determined compared to the reference gene hypoxanthine-guanine phosphoribosyltransferase by real-time quantitative PCR.[20 21] We previously showed that lung cytokine messenger RNA (mRNA) levels correlate with protein levels.[22]

ELISA

Concentrations of cytokines were determined in Ova- (interleukin (IL)-5, IL-13 and TNF-α) or *Hi*- (IL-17) stimulated mediastinal lymph node (MLN) culture supernatants by ELISA (R&D systems, Minnesota, USA).[18]

Statistics

Data are represented as mean±SEM with 6–12 mice in each group. Statistical significance was determined by one-way analysis of variance (ANOVA) with Tukey's or Fisher's least significant difference post-tests. AHR was analysed using two-way repeated measures ANOVA with Tukey's post-test. (GraphPad Prism V.6 Software, San Diego, California, USA). See online supplement for tables of full statistical comparisons of all groups.

RESULTS

Infection-induced SSIAAD

To investigate the effects of antibiotics, we developed a novel model of *Chlamydia*-induced SSI asthma, and compared it with our model of *Hi*-induced SSI asthma (figure 1). Treatment groups received antibiotics on d17–21 (3–7 days post infection), and/or DEX on d32–34 (figure 1A, *Chlamydia*), or antibiotics on d-8 to d-6 and/or DEX on d13–15 (figure 1B, *Hi*).

Ova-induced AAD (Ova groups) was characterised by increased airway inflammation, Th2 responses and AHR compared with non-allergic (saline) controls (figures 2–7). DEX alone treatment significantly reduced all these cardinal features of AAD. *Chlamydia* infection (Ova/Cmu), however, increased airway neutrophils and TNF-α responses and decreased eosinophils and Th2 responses compared with uninfected controls (Ova). Inflammation and AHR were insensitive to DEX treatment.

We next assessed the effect of amoxicillin and clarithromycin treatment on steroid-sensitive AAD and *Chlamydia-induced* SSIAAD in order to delineate between the antimicrobial and anti-inflammatory effects of macrolide therapy.

Clarithromycin, but not amoxicillin, treatment broadly suppressed the cardinal features of steroid-sensitive AAD

First, we assessed the effects of amoxicillin, an antibiotic with only antimicrobial properties, and clarithromycin, an antibiotic with both antimicrobial and anti-inflammatory properties, on steroid-sensitive AAD (Ova groups).

Amoxicillin treatment (Ova/Amox) did not alter total leucocyte numbers, but reduced neutrophils and eosinophils and, surprisingly, increased macrophage numbers in BALF compared with untreated controls (Ova, figure 2A–D). Treatment had no effect on AHR (airways resistance, figure 2E). Amoxicillin had no additive beneficial effects with DEX treatment (Ova/Amox/Dex), which reduced airway total cells, neutrophils, eosinophils, macrophages and airways resistance.

Clarithromycin treatment alone (Ova/Clari) significantly reduced total cells, neutrophils and eosinophils in BALF as well as AHR (figure 2A–E). Similar reductions also occurred with clarithromycin in combination with DEX (Ova/Clari/Dex).

Clarithromycin, but not amoxicillin, treatment broadly suppressed the cardinal features of *Chlamydia*-induced SSIAAD

Next, we assessed the effect of amoxicillin and clarithromycin treatments on *Chlamydia*-induced SSIAAD. We first showed that 3–10 mg/kg doses of both antibiotics had the same effects on infection (see online supplementary figure S1A). Thus, 5 mg/kg of either clarithromycin or amoxicillin was administered on d17–21 of the model (figure 1A).

Amoxicillin treatment alone in SSIAAD (Ova/Cmu/Amox) reduced BALF neutrophils and eosinophils, but had no effect on total cells or macrophages compared with untreated controls (Ova/Cmu, figure 3A–D). Treatment again had no effect on AHR (figure 3E). When amoxicillin was combined with DEX (Ova/Cmu/Amox/Dex), treatment resulted in reductions in all BALF cells and AHR. Since DEX alone did not suppress total cell, neutrophil, eosinophil or macrophage numbers in BALF or AHR in SSIAAD, it is likely that amoxicillin restores sensitivity to DEX.

In contrast to amoxicillin, clarithromycin treatment (Ova/Cmu/Clari) reduced total cells, neutrophils and macrophages in BALF as well as AHR (figure 3A–E). When clarithromycin was combined with DEX (Ova/Cmu/Clari/DEX), treatment suppressed total leucocytes, eosinophils, macrophages and AHR.

Thus, amoxicillin alone is unable to broadly suppress inflammation and AHR but does restore steroid-sensitivity in *Chlamydia*-induced SSIAAD. Clarithromycin alone broadly suppresses steroid-sensitive AAD and SSIAAD.

Amoxicillin, but not Clarithromycin, inhibits steroid-induced reactivation of infection

We then assessed the effect of antibiotic treatment on the persistence/reactivation of infection, by determination of *Cmu* 16S expression in whole lung tissue.[23] The infection is typically cleared within 20 days in non-allergic mice (ie, before the second Ova challenges on d32).[20] 16S levels were low/undetectable in amoxicillin and clarithromycin-treated (Ova/Cmu/Amox and Ova/Cmu/Clari) or untreated groups with SSIAAD (Ova/Cmu; figure 3F). Notably, DEX treatment reactivated the infection in SSIAAD (Ova/Cmu/DEX), but this was prevented in groups that were treated in combination with amoxicillin (Ova/Cmu/Amox/DEX). However, reactivation of infection with DEX was not prevented by clarithromycin.

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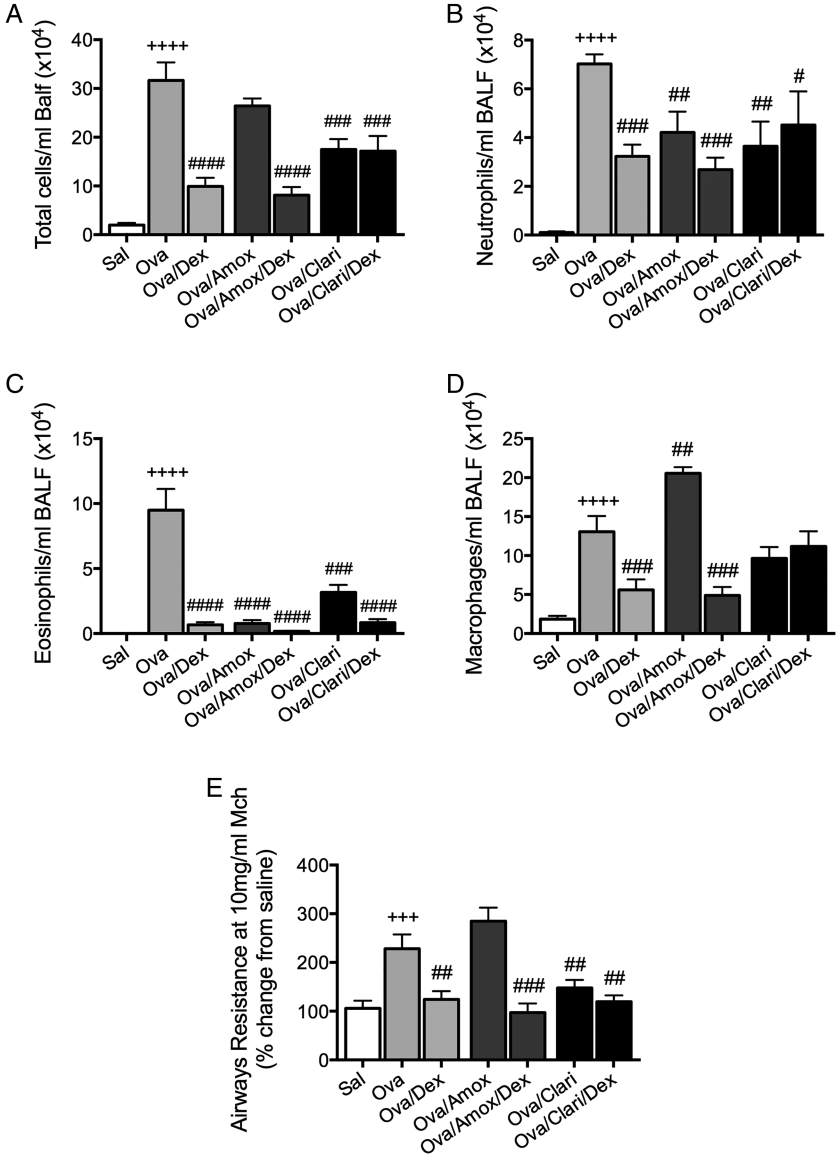

Figure 2 Amoxicillin treatment suppresses some features of steroid-sensitive allergic airways disease and clarithromycin suppresses all features of disease. Effects of amoxicillin and clarithromycin treatment on total cells (A), neutrophils (B), eosinophils (C) and macrophages (D) in bronchoalveolar lavage fluid of steroid-sensitive groups were assessed. Airways hyper-responsiveness in terms of airways resistance at the maximal dose of methacholine (10 mg/mL, E) was also assessed. Results are representative of two independent experiments, with a total of five to eight mice/group and all data are presented as means±SEM. $^{+++}$ $^{+}$p<0.0001, $^{+++}$p<0.001 compared with non-allergic (Sal) controls, $^{\#\#\#\#}$p<0.0001, $^{\#\#\#}$p<0.001, $^{\#\#}$p<0.01, $^{\#}$p<0.05 compared with allergic (Ova) controls.

Clarithromycin, but not amoxicillin, broadly suppresses inflammatory mediators in steroid-sensitive AAD and *Chlamydia*-induced SSIAAD

To investigate the effect of antibiotic treatment on inflammatory mediator responses, the levels of protein (in MLN culture supernatants) and mRNA expression (in lung tissue) of IL-5, IL-13 and TNF-α were assessed. In steroid-sensitive AAD, amoxicillin decreased the protein levels of IL-13, but had no effect on IL-5 (protein or mRNA), IL-13 mRNA or TNF-α (protein or mRNA; figure 4A–C and G–I). In combination with DEX, amoxicillin also decreased IL-5 and TNF-α protein and mRNA. Clarithromycin significantly reduced IL-5, IL-13 and TNF-α protein and IL-13 mRNA. In combination with DEX, the suppressive effects on TNF-α protein and 13 mRNA were removed.

In SSIAAD, amoxicillin decreased IL-13 protein, but increased IL-5 and IL-13 mRNA, and restored the sensitivity of TNF-α mRNA to DEX (figure 4D–F and J–L). Clarithromycin suppressed TNF-α release and mRNA. This also occurred in combination with DEX although IL-13 mRNA was increased.

These results demonstrate that amoxicillin does not broadly reduce inflammatory cytokine responses in steroid-sensitive or SSIAAD. In contrast, clarithromycin alone suppressed these inflammatory cytokine responses in steroid-sensitive AAD and reduced TNF-α responses in SSIAAD. These findings indicate that TNF-α may be an important factor in driving SSIAAD that is suppressed by clarithromycin treatment.

Anti-TNF-α therapy suppresses *Chlamydia*-induced SSIAAD

To further investigate the role of TNF-α in SSIAAD, the effects of anti-TNF-α antibody treatment were assessed. Treatment reduced total cells, neutrophils, eosinophils and macrophages in BALF as well as AHR in SSIAAD (Ova/Cmu/anti-TNF-α) back to levels in uninfected allergic controls (Sal/Cmu, figure 5A–E). These results show that TNF-α plays a crucial pathogenic role in *Chlamydia*-induced SSIAAD.

Clarithromycin suppresses *Hi*-induced SSIAAD

We have previously shown that *Hi* infection induces SSIAAD driven by Th17 responses.[10] To determine whether clarithromycin has widespread applicability for SSI asthma, we assessed its efficacy in our *Hi*-induced model. Clarithromycin significantly reduced *Hi* levels at the peak of infection (see online supplementary figure S1B). In *Hi*-induced SSIAAD (Hi/Ova), clarithromycin treatment reduced neutrophils in BALF as well as AHR,

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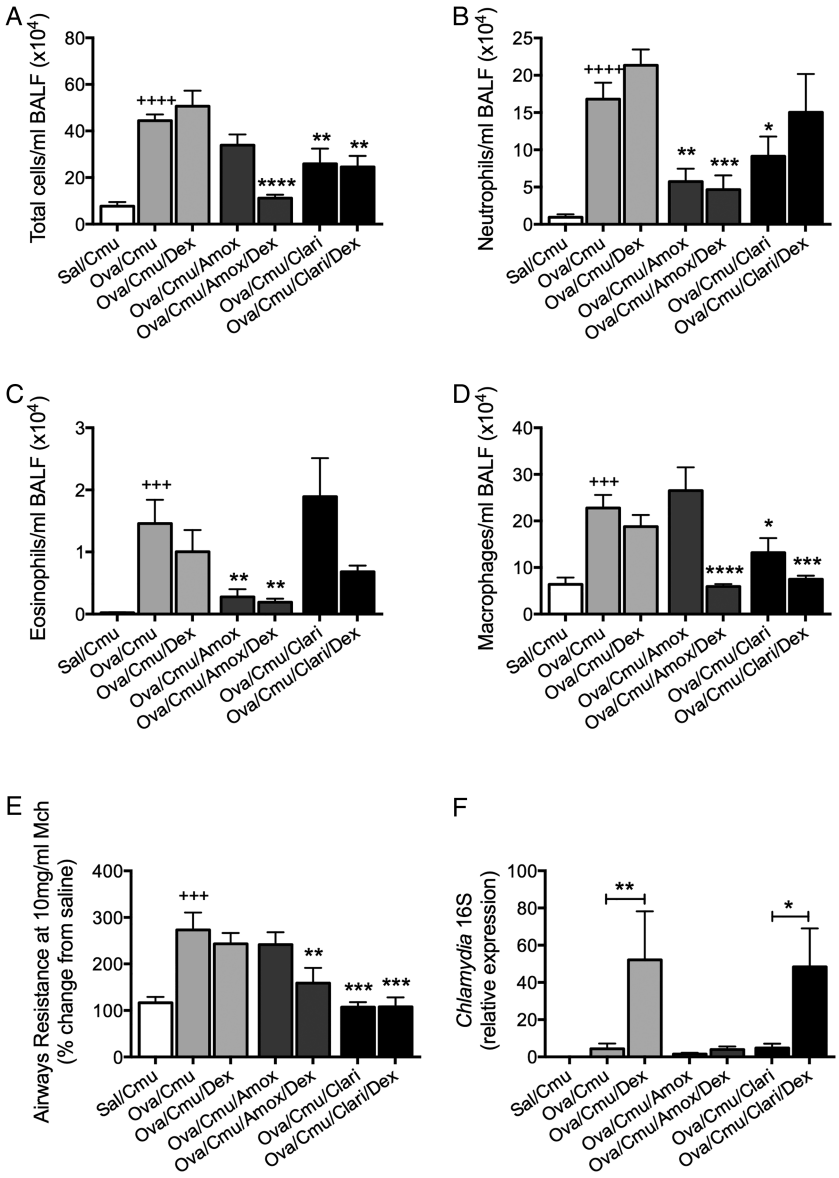

Figure 3 Clarithromycin, but not amoxicillin, treatment broadly suppresses *Chlamydia*-induced, severe, steroid-insensitive neutrophilic allergic airways disease (SSIAAD). Effects of amoxicillin and clarithromycin treatment on total cells (A), neutrophils (B), eosinophils (C) and macrophages (D) in bronchoalveolar lavage fluid of SSIAAD groups were assessed. Airways hyper-responsiveness in terms of airways resistance at the maximal dose of methacholine (10 mg/mL, E) and *Chlamydia* 16S RNA levels in the lungs (F) were also assessed. Results are representative of two independent experiments, with a total of five to eight mice/group, and all data are presented as means±SEM. $^{++++}$p<0.0001, $^{+++}$p<0.001 compared with non-allergic (Sal/Cmu) controls, ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 compared with infected allergic (Ova/Cmu) groups.

had no effect on total cells, but increased BALF eosinophils compared with untreated controls (figure 6A–E). However, clarithromycin in combination with DEX significantly reduced total cells, neutrophils and eosinophils, had no effect on macrophages, but suppressed AHR.

Clarithromycin suppresses IL-17 responses in *Hi*-induced SSIAAD

In *Hi*-induced SSIAAD, clarithromycin increased IL-5 and IL-13, but the addition of DEX reduced the levels of these factors back to those in untreated controls (figure 7A, B). Importantly, clarithromycin alone and in combination with DEX significantly reduced IL-17 levels (figure 7C).

Thus, clarithromycin suppresses SSIAAD and reverses steroid-insensitivity induced by a different infection in a different protocol, by reducing the Th17 responses that drive that phenotype.

DISCUSSION

Twenty per cent of asthmatics have neutrophilic rather than eosinophilic asthma. Neutrophilic asthma is more resistant to anti-inflammatory corticosteroid treatment and is difficult to manage.[24] The mechanisms of pathogenesis of this asthma endotype are poorly understood and effective therapies remain to be defined. We previously showed that *Chlamydia* and *Hi* infections that are associated with this endotype induce a switch from an eosinophilic, Th2-mediated disease to a neutrophilic, Th1 and/or Th17-mediated phenotype with suppressed Th2 responses in AAD.[10 18 21] Here, we show that these factors are involved in the pathogenesis of steroid-insensitivity and highlight novel therapeutic strategies.

Asthmatics are more susceptible to infection and this may drive SSI asthma. To better model this scenario, we first developed a novel model of *Chlamydia*-induced SSI asthma. This model involves the induction of a steroid-insensitive phenotype by infection in established AAD. Here, we demonstrate that amoxicillin alone is able to clear infection in SSIAAD, but does not broadly reduce BALF inflammation, T cell cytokine responses or AHR. It does suppress some features, eosinophils and neutrophils are reduced, which may be specifically modulated by amoxicillin in steroid-sensitive AAD. Interestingly, amoxicillin treatment did restore steroid-sensitivity of airway inflammation and AHR in SSIAAD. This is likely to involve the reduction in infection-induced neutrophils, which we have

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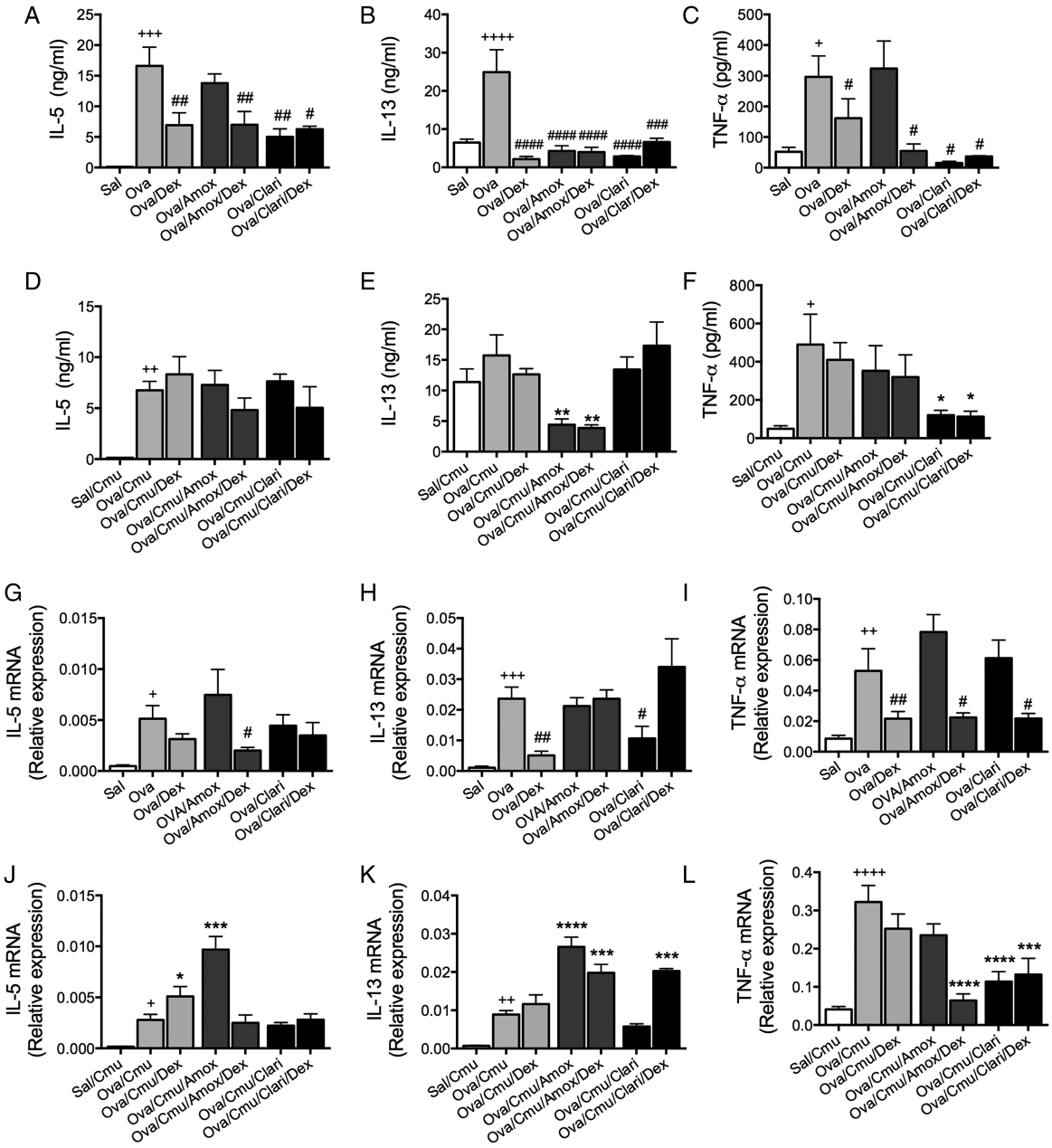

Figure 4 Clathrithromycin, but not amoxicillin, treatment broadly suppresses important inflammatory mediators in steroid-sensitive and *Chlamydia*-induced, severe, steroid-insensitive, neutrophilic allergic airways disease (SSIAAD). Effects of amoxicillin and clarithromycin treatment on interleukin (IL)-5 (A), IL-13 (B) and tumour necrosis factor α (TNF-α; C) protein release from mediastinal lymph nodes in steroid-sensitive groups, and IL-5 (D), IL-13 (E) TNF-α (F) protein in SSIAAD groups were assessed. Effects of treatments on lung mRNA expression of IL-5, IL-13 and TNF-α in steroid-sensitive (G–I) and SSIAAD groups (J–L) were also assessed. Results are representative of two independent experiments, with a total of 5–10 mice/group, and all data are presented as means±SEM. $^{++++}p<0.0001$, $^{+++}p<0.001$, $^{++}p<0.01$, $^{+}p<0.05$ compared with non-allergic (Sal, or Sal/Cmu) controls, $^{\#\#\#\#}p<0.0001$, $^{\#\#\#}p<0.001$, $^{\#\#}p<0.01$, $^{\#}p<0.05$ compared with allergic (Ova) controls, ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 compared with infected, allergic (Ova/Cmu) groups.

previously shown to be important in the development of severe neutrophilic AAD.[21] However, with infection and neutrophils suppressed, there is still underlying Ova-induced AAD and therefore, DEX treatment is still required in order to suppress the remaining features of disease, including leucocytes in BALF and AHR. We also showed that DEX treatment reactivates *Chlamydia* infection that is inhibited by amoxicillin. In contrast, clarithromycin treatment alone substantially reduced airway inflammation, T cell responses, AHR and infection in two different models of SSIAAD, with similar effects when combined with DEX. This indicates that clarithromycin could be an effective therapy for steroid-sensitive and SSI asthma.

Studies of steroid-insensitive patients on high doses of steroids have shown that they have no reductions in IL-4 and IL-5 expression in BAL cells, and that there is poor suppression of cytokine and chemokine release from peripheral monocytes and alveolar macrophages.[25–28] Significantly, our data showing that steroids reactivate an infection suggest that they may be detrimental in infection-induced SSIAAD. We have previously shown that DEX reactivates *Hi* infection in AAD,[10] and extend these

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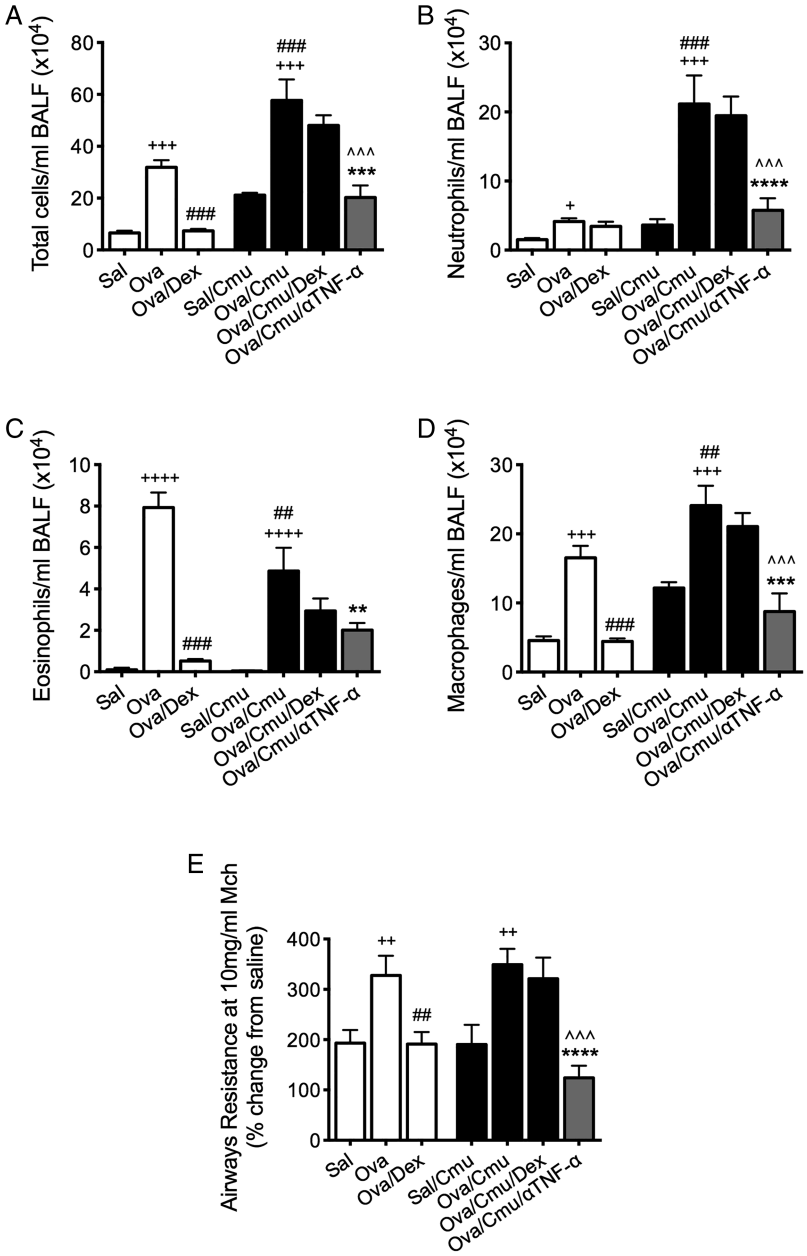

Figure 5 Anti-tumour necrosis factor α (anti-TNF-α) therapy suppresses *Chlamydia*-induced, severe, steroid-insensitive, neutrophilic allergic airways disease. Effects of anti-TNF-α treatment on total cells (A), neutrophils (B), eosinophils (C) and macrophages (D) in bronchoalveolar lavage fluid (BALF). Airways hyper-responsiveness in terms of airways resistance at the maximal dose of methacholine (10 mg/mL, E) was also assessed. Results are representative of one experiment with 4–10 mice per group, and all data are presented as means ±SEM. $^{+++}p<0.001$, $^{++}p<0.01$, $^{+}p<0.05$ compared with non-allergic (Sal, or Sal/Cmu) controls, $^{###}p<0.001$, $^{##}p<0.01$, $^{#}p<0.05$ compared with allergic (Ova) controls, $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ compared with infected, allergic (Ova/Cmu) groups, $^{\wedge\wedge\wedge}p<0.001$, $^{\wedge}p<0.05$ compared with infected, allergic, dexamethasone (DEX)-treated (Ova/Cmu/Dex) groups.

studies here by demonstrating that *Chlamydia* infection is also reactivated. These studies suggest that alternative therapies are required for steroid-insensitive patients. The mechanisms of steroid-induced reactivation of infection have not been investigated. They are likely due to the suppressive effects of DEX on immune/inflammatory responses that are needed to clear infection, thereby creating an environment where bacteria can proliferate.

Macrolides have been proposed as new therapies for SSI asthma as they have both antimicrobial and anti-inflammatory properties and are in widespread clinical use. However, the relative contributions of these properties to efficacy are not known. To date experimental studies of macrolides have been limited to models of mild-to-moderate steroid-sensitive AAD. To delineate the relevant contributions, we compared the effects of amoxicillin, a purely antibacterial agent, with clarithromycin, an antibiotic with additional anti-inflammatory properties in SSIAAD. Although amoxicillin treatment suppressed infection to the same extent as clarithromycin, it only reduced granulocytic inflammation, IL-13 protein, but not other inflammatory mediators and had no effect on AHR in both steroid-sensitive and SSIAAD. Amoxicillin did restore steroid-sensitivity in steroid-insensitive groups. In contrast, clarithromycin treatment alone broadly reduced both inflammation and AHR in both endotypes. Few clinical studies have examined the effect of macrolide treatment in asthma with evidence of infection, however, those that have been performed have had some success. Treatment of adult asthmatics with evidence of *Cpn* infection with roxithromycin for 6 weeks, significantly improved lung function.[29] In asthmatic children with *Cpn* infection, clarithromycin reduced the duration and risk of subsequent wheezing episodes.[30] Other studies have also shown that macrolide treatment reduces inflammation in asthma. Kraft *et al*[31] showed, in asthmatics that were PCR-positive for *Cpn*, that clarithromycin treatment reduced IL-5 levels. Despite these data, it remains unclear how macrolides exert their beneficial effects.

We investigated likely mechanisms and immune responses involved in the suppression of disease by clarithromycin. In

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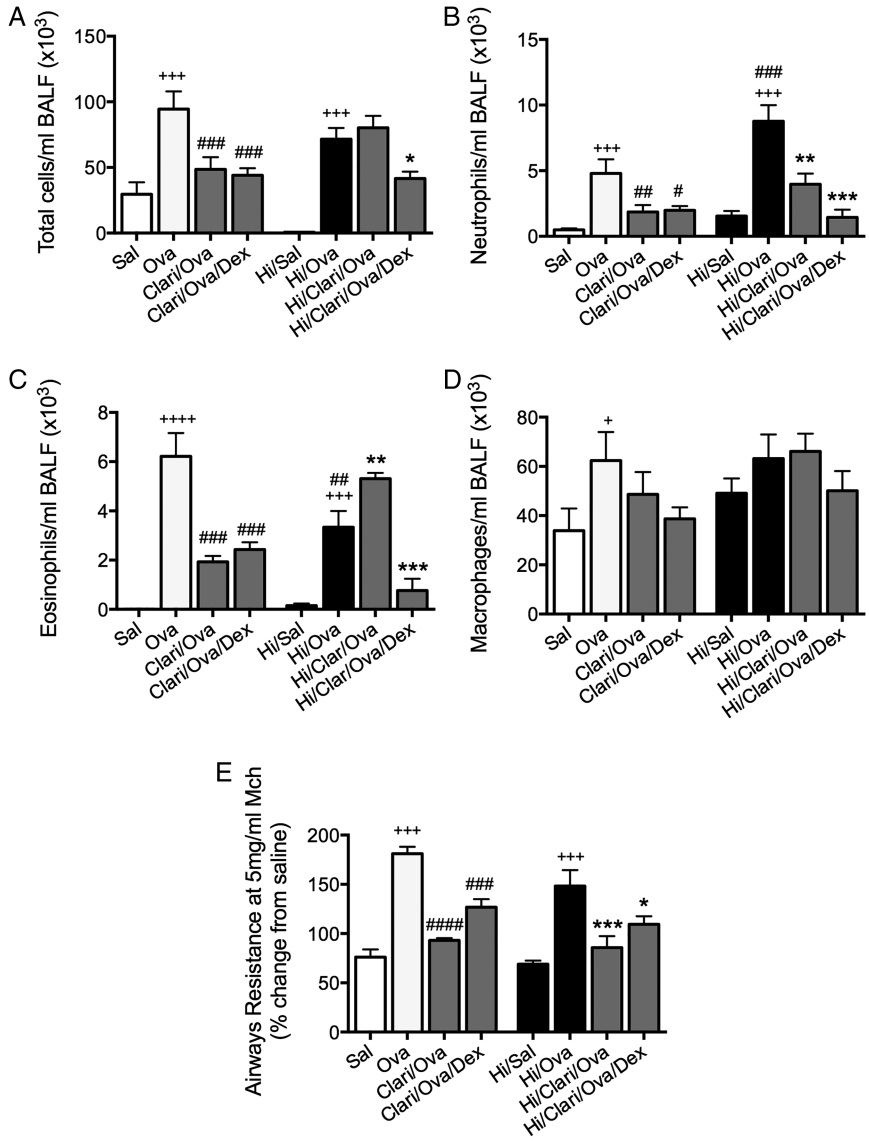

Figure 6 Clarithromycin suppresses *Haemophilus influenzae* (*Hi*)-induced, severe, steroid-insensitive, neutrophilic allergic airways disease. Effects of clarithromycin treatment on total cells (A), neutrophils (B), eosinophils (C) and macrophages (D) in bronchoalveolar lavage fluid(BALF). Airways hyper-responsiveness in terms of airways resistance at the maximal dose of methacholine (5 mg/mL, E) was also assessed. Results are representative of two independent experiments, with a total of six mice/group, and all data are presented as means±SEM. $^{++++}$p<0.0001, $^{+++}$p<0.001, $^{+}$p<0.05 compared with non-allergic (Sal, or Hi/Sal) controls, $^{\#\#\#\#}$p<0.0001, $^{\#\#\#}$p<0.001, $^{\#\#}$p<0.01, $^{\#}$p<0.05 compared with allergic (Ova) controls, ***p<0.001, **p<0.01, *p<0.05 compared with infected, allergic (Hi/Ova) groups.

steroid-sensitive groups, clarithromycin reduced both IL-5 and IL-13, which are important in Th2-mediated asthma.[24 32] Clarithromycin-induced reductions in these cytokines likely suppress the recruitment of eosinophils to the airways, mucus production and AHR. Trials using anti-IL-5 antibodies such as reslizumab, reduced asthma exacerbations and improved FEV_1 compared with placebo groups.[24 32] A recent trial using lebrikizumab, an anti-IL-13 antibody, showed significant reductions in FEV_1 after treatment.[32] We showed that IL-13 responses promote bacterial infections and associated SSIAAD,[18 22 33] and macrolides may also have beneficial effects in reducing this susceptibility.

In steroid-insensitive groups, clarithromycin treatment, alone and combined with DEX, reduced TNF-α production. To further investigate its role, we inhibited this cytokine during SSIAAD using monoclonal antibodies. Inhibition of TNF-α suppressed neutrophil and macrophage infiltration and AHR, and in the presence of DEX, also reduced eosinophils. This suggests that TNF-α is crucial for the induction of this asthma endotype, and its suppression by clarithromycin reduced key features of disease. Another recent study by Manni *et al*[34] investigated SSIAAD induced by the adoptive transfer of Th17 cells to show that anti-TNF-α treatment reduced lung compliance and airway inflammation, but not tissue inflammation or AHR. In contrast, infection-induced SSIAAD is not solely driven by IL-17. These studies are clinically relevant as TNF-α is a pro-inflammatory cytokine that is implicated in the pathogenesis of severe asthma.[32] The administration of anti-TNF-α antibodies to patients with SSI asthma led to improved lung function and AHR, and quality of life.[35] However, there have been concerns about its safety.[36] We show that TNF-α responses were substantially increased in SSIAAD, which is in agreement with studies that suggest that anti-TNF-α treatment may be most beneficial in SSI asthma.

Together, our studies show that clarithromycin targets Th2 responses in steroid-sensitive AAD, and TNF-α responses in *Chlamydia*-induced SSIAAD. Thus, this macrolide may have widespread applicability in the treatment of asthma.

This was further confirmed when we investigated the effects of clarithromycin in a different model of infection-induced SSI asthma. We previously showed that *Hi* infection also induces an SSIAAD phenotype.[18] We show in this model that clarithromycin increased Th2 responses, including IL-5, IL-13 and eosinophils. We suggest that this may occur as *Hi* infection reduces these responses, and when macrolide treatment suppresses infection, the inhibitory effects on these features are removed.

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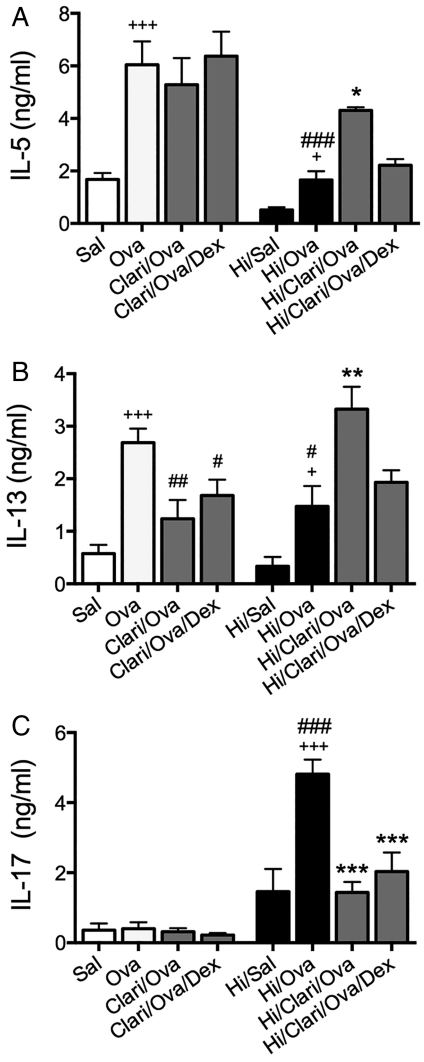

Figure 7 Clarithromycin suppresses interleukin (IL)-17 responses in *Haemophilus influenzae* (*Hi*)-induced, severe, steroid-insensitive, neutrophilic allergic airways disease. Effects of clarithromycin treatment on IL-5 (A), IL-13 (B), and IL-17 (C) release from mediastinal lymph nodes. Results are representative of two independent experiments, with a total of six mice/group, and all data are presented as means±SEM. $^{+++}$p<0.001, $^{+}$p<0.05 compared with non-allergic (Sal, or Hi/Sal) controls, $^{###}$p<0.001, $^{##}$p<0.01, $^{#}$p<0.05 compared with allergic (Ova) controls, ***p<0.001, **p<0.01, *p<0.05 compared with infected, allergic (Hi/Ova) groups.

Combined treatment with clarithromycin and DEX had the same effects as treatment in the *Chlamydia*-induced phenotype and significantly reduced neutrophils, eosinophils and AHR. Importantly, we also showed that clarithromycin significantly reduced IL-17 responses in *Hi*-induced SSIAAD. We have previously shown that this *Hi*-induced phenotype is mediated by IL-17 responses and that inhibiting this cytokine with monoclonal antibodies reduced key features of disease.[10] Thus, clarithromycin may be effective in this phenotype by suppressing IL-17 production. In a similar manner, our clinical studies have shown that clarithromycin treatment reduced airway neutrophil numbers, and sputum IL-8, neutrophil elastase and matrix metalloproteinase-9 levels in SSI asthma.[37]

Numerous studies have shown that the immunomodulatory activities of macrolides result from their accumulation inside inflammatory cells and suppress ERK1/2 phosphorylation and NF-κB activation that attenuates inflammatory responses in these cells. This is likely how clarithromycin is suppressing SSIAAD in our studies.[38]

In conclusion, this study demonstrates that clarithromycin has antimicrobial as well as broad immunomodulatory effects and reduces the dominant inflammatory mediators (eg, TNF-α, IL-17) that drive the different endotypes of AAD. In steroid-sensitive groups, clarithromycin suppressed IL-13 to reduce disease features, whereas in a TNF-α or IL-17 environment, it suppressed these factors to reduce disease features. This study, therefore, shows how clarithromycin may be working to suppress key features of infection-induced SSIAAD. Importantly, these studies have identified the efficacy of this treatment on inflammation and infection, and further promote its use as a therapy for infection-induced SSI asthma.

Contributors The work presented here was performed in collaboration with all authors. A-TE and JCH: Hypothesis, conception, design, performance and interpretation of experiments, establishment of in vivo models and writing the manuscript. RYK, JWP, ELB and MRS: Acquisition of in vivo data and revision of the manuscript. JRM: Establishment of in vivo models. JLS, PSF and PGG: Conception, revision of the manuscript. PMH: Senior author and responsible for the overall content, hypothesis, conception, design, interpretation of data and revision of the manuscript.

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Competing interests None.

Ethics approval All experiments were approved by the Animal Ethics Committee, University of Newcastle.

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Macrolide therapy suppresses key features of experimental steroid-sensitive and steroid-insensitive asthma

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Methods

Experimental models

A novel model of *Chlamydia* respiratory infection-induced, severe, steroid-insensitive, neutrophilic allergic airway disease (SSIAAD) was developed to reproduce the effects of infection in established asthma. Female (6-8 week-old) BALB/c mice were intraperitoneally (IP) sensitised to Ova (50µg [Sigma-Aldrich, Castle Hill, Australia]), in the Th2-inducing adjuvant alum (1mg Rehydrogel [Reheis, Berkeley Heights, NJ] in 200µl 0.9% saline) on day 0 [1]. Mice were then intranasally (IN) challenged with Ova on d12-13 and d33-34 (10µg; 50µl sterile saline [Figure 1A]). On d14 mice were inoculated IN with the natural mouse pathogen *C. muridarum* (Cmu; 100 inclusion-forming units, ATCCVR-123, 30µl sucrose phosphate glutamate buffer [SPG]).[1, 2] Dexamethasone (DEX) was administered IN (2mg/kg; 50µl phosphate buffered saline [PBS]) on d32-34 with the Ova challenges (Figure 1A).

We also investigated the broader applicability of our data in a different, previously developed model using *Haemophilus influenzae* (*Hi*) infection. Mice were inoculated intratracheally (IT) with $5x10^5$ colony-forming units (CFU) non-typeable *Hi* (NTHi-289, 30ul PBS) 10 days prior (d-10) to Ova IP sensitisation (50µg; 200µl in alum 0.9% saline). They were then challenged with Ova (10µg; 50µl sterile saline) on d12-15 (Figure 1B). DEX (1mg/kg; 50µl PBS) was administered IN on d13-15.[3]

All controls were sham-sensitised with saline, and sham-inoculated with SPG (*Chlamydia*-induced AAD) or PBS (*Hi*-induced AAD). In both models, mice were sacrificed 24h after the final challenge and features of AAD were assessed.

Lung function

Two methods of lung function measurement were used to assess the robustness of the effects of antibiotic treatment in the two different models. Lung function for all the *Chlamydia* groups (and their controls) was analysed using the FlexiVent system. Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg, Troy Laboratories, Smithfield, Australia) and their tracheas were cannulated. FlexiVent apparatus (FX1 System; SCIREQ, Montreal, Canada) was used to assess airways-specific resistance (tidal volume of 8 mL/kg and respiratory rate of 450 breaths/min). Three measurements per dose were taken in response to increasing doses of nebulized methacholine (Sigma-Aldrich, Sydney, Australia) and the average calculated. Lung function for all the *Haemophilus* groups and their controls was analysed using the Buxco R&C system. Mice were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg) and their tracheas were cannulated. R&C apparatus (BUXCO Electronics, Sharon, CT, USA) was used to assess airways resistance (at a tidal volume of 9 mL/kg and respiratory rate of 180 breaths/min). Peak airways resistance was determined in response to increasing doses of nebulized methacholine (Sigma-Aldrich).[1, 3]

Lung mRNA expression

RNA was extracted from homogenised whole lung tissue using TRIzol® according to manufacturer's instructions (Invitrogen, Mount Waverly, Australia) and reverse-transcribed using BioScript™ (Bioline Pty. Ltd., NSW, Australia) and random hexamer primers (Invitrogen, Life Technologies, Australia). Relative abundance of cytokine cDNA was determined compared to the reference gene hypoxanthine-guanine phosphoribosyltransferase (HPRT) by real-time PCR (Mastercycler® ep

realplex[2] system; Eppendorf South Pacific Pty. Ltd., NSW, Australia).[1, 4] Primers used were HPRT Fwd 5'- aggccagactttgttggatttgaa-3', Rev 5'-caacttgcgctcatcttaggcttt-3'; IL-13 Fwd 5'-tgcttgccttggtggtct-3', Rev 5'-ggggagtctggtcttgtgtg-3'; IL-5 Fwd 5'-catcacaccaaggaactcttgcag-3', Rev 5'-tggtgaaagagaccttgacacagc-3'; TNF-α Fwd 5'-tctgtctactgaacttcggggtga-3', Rev 5'-ttgtctttgagatccatgccgtt-3'; and Cmu 16s Fwd 5'-gcggcagaaatgtcgtttt-3', Rev 3'-cgctcgttgcgggactta-5'.

Mediastinal lymph node (MLN) T-cell cytokine release

MLN cells ($5x10^6$ cells) were isolated, re-stimulated with Ova (200 mg/mL; Sigma) or ethanol-killed *Hi* ($2x10^7$ CFU) and cultured for 6 days in Gibco RPMI-1640 (Invitrogen) containing 10% FCS, 20 mmol/L HEPES, 10 mg/mL penicillin/streptomycin, 2 mmol/L L-glutamine, and 50 mmol/L 2-mercaptoethanol.[5]

Statistics

Data are represented as mean ± SEM with 6-12 mice in each group. For all data represented in histograms, statistical significance was determined by one-way analysis of variance (ANOVA) with a Tukey's or Fisher's LSD post-test. AHR was analysed using two-way repeated measures ANOVA with a Tukey's post-test. (GraphPad Prism 6 Software, San Diego, California, USA).

Discussion

Use of structurally related macrolides that are non-antibiotic as a control

We considered using a number of different compounds, including rapamycin, tacrolimus and pimecrolimus, which are all structurally related to clarithromycin.

Rapamycin has been shown to reduce disease features in AAD during the induction phase, but in established disease, exacerbates clinical features. We concluded that using this molecule would complicate our study and make it difficult to interpret the outcomes. Tacrolimus has been shown to interfere with the early events of *Chlamydia* infection *in vitro*. Finally, pimecrolimus has been shown to inhibit the growth of *Malassezia* species of fungi. Thus both of these compounds have anti-microbial effects. We, therefore, have not been able to find a structurally related macrolide that does not have antibiotic properties.

IL-5 increases in clarithromycin-treated groups with *Hi*-induced SSIAAD (Figure 7)

We show that clarithromycin and DEX treatment does not inhibit IL-5 in groups with either the steroid-sensitive (Clari/Ova/Dex) or *Hi*-induced SSIAAD (Hi/Clari/Ova/Dex) compared to their untreated controls (Ova or Hi/Ova). *Hi* infection in AAD (Hi/Ova) reduces IL-5 responses almost down to baseline levels. Clarithromycin and DEX treatment of this group (Hi/Clari/Ova/Dex) does not decrease IL-5 any further. In contrast IL-5 increases in *Hi*-induced SSIAAD with clarithromycin treatment (Hi/Clari/Ova) compared to untreated controls (Hi/Ova). This is likely to result from the removal of the suppressive effects of *Hi* infection on IL-5 by clarithromycin.

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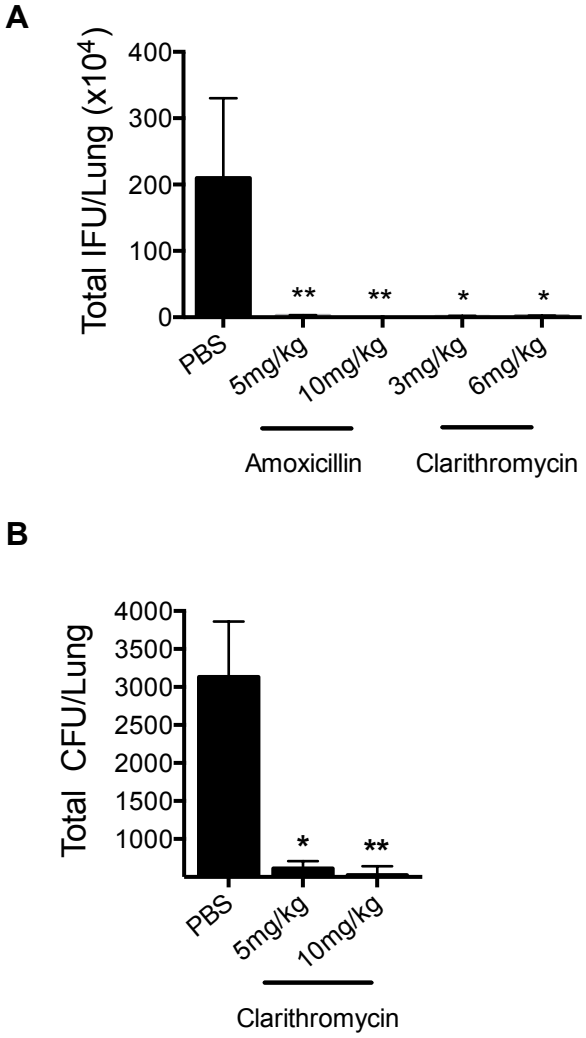


Supplementary Figure 1: Equipotent doses of antibiotics had the same effects on *Chlamydia* and *Hi* infection.

A

One way ANOVA (Total cells)	Mean Diff.	Summary	P Value
Sal vs. Ova	-29.66	****	< 0.0001
Sal vs. Ova/Dex	-7.919	**	0.0058
Sal vs. Ova/Clari	-15.5	****	< 0.0001
Sal vs. Ova/Clari/Dex	-15.16	****	< 0.0001
Sal vs. Ova/Amox	-24.42	****	< 0.0001
Sal vs. Ova/Amox/Dex	-6.136	*	0.0369
Ova vs. Ova/Dex	21.74	****	< 0.0001
Ova vs. Ova/Clari	14.16	***	0.0001
Ova vs. Ova/Clari/Dex	14.5	****	< 0.0001
Ova vs. Ova/Amox	5.237	ns	0.1002
Ova vs. Ova/Amox/Dex	23.52	****	< 0.0001
Ova/Dex vs. Ova/Clari	-7.585	*	0.0155
Ova/Dex vs. Ova/Clari/Dex	-7.243	*	0.0205
Ova/Dex vs. Ova/Amox	-16.5	****	< 0.0001
Ova/Dex vs. Ova/Amox/Dex	1.784	ns	0.5553
Ova/Clari vs. Ova/Clari/Dex	0.3416	ns	0.9131
Ova/Clari vs. Ova/Amox	-8.919	**	0.0066
Ova/Clari vs. Ova/Amox/Dex	9.368	**	0.0045
Ova/Clari/Dex vs. Ova/Amox	-9.26	**	0.005
Ova/Clari/Dex vs. Ova/Amox/Dex	9.027	**	0.0061
Ova/Amox vs. Ova/Amox/Dex	18.29	****	< 0.0001

B

One way ANOVA (Neutrophils)	Mean Diff.	Summary	P Value
Sal vs. Ova	-6.929	****	< 0.0001
Sal vs. Ova/Dex	-3.139	**	0.002
Sal vs. Ova/Clari	-3.552	***	0.0009
Sal vs. Ova/Clari/Dex	-4.421	***	0.0001
Sal vs. Ova/Amox	-4.118	***	0.0002
Sal vs. Ova/Amox/Dex	-2.591	*	0.0122
Ova vs. Ova/Dex	3.79	***	0.0005
Ova vs. Ova/Clari	3.377	**	0.0021
Ova vs. Ova/Clari/Dex	2.508	*	0.0246
Ova vs. Ova/Amox	2.811	**	0.0091
Ova vs. Ova/Amox/Dex	4.339	***	0.0001
Ova/Dex vs. Ova/Clari	-0.4132	ns	0.6764
Ova/Dex vs. Ova/Clari/Dex	-1.282	ns	0.2228
Ova/Dex vs. Ova/Amox	-0.9792	ns	0.3254
Ova/Dex vs. Ova/Amox/Dex	0.5483	ns	0.5801
Ova/Clari vs. Ova/Clari/Dex	-0.869	ns	0.4216
Ova/Clari vs. Ova/Amox	-0.5659	ns	0.5821
Ova/Clari vs. Ova/Amox/Dex	0.9616	ns	0.3517
Ova/Clari/Dex vs. Ova/Amox	0.3031	ns	0.7784
Ova/Clari/Dex vs. Ova/Amox/Dex	1.831	ns	0.0954
Ova/Amox vs. Ova/Amox/Dex	1.528	ns	0.1427

C

One way ANOVA (Eosinophils)	Mean Diff.	Summary	P Value
Sal vs. Ova	-9.495	****	< 0.0001
Sal vs. Ova/Dex	-0.6594	ns	0.3838
Sal vs. Ova/Clari	-3.165	***	0.0002
Sal vs. Ova/Clari/Dex	-0.834	ns	0.324
Sal vs. Ova/Amox	-0.771	ns	0.3326
Sal vs. Ova/Amox/Dex	-0.1742	ns	0.8257
Ova vs. Ova/Dex	8.835	****	< 0.0001
Ova vs. Ova/Clari	6.329	***	0.0001
Ova vs. Ova/Clari/Dex	8.661	****	< 0.0001
Ova vs. Ova/Amox	8.724	****	< 0.0001
Ova vs. Ova/Amox/Dex	9.321	****	< 0.0001
Ova/Dex vs. Ova/Clari	-2.506	**	0.0059
Ova/Dex vs. Ova/Clari/Dex	-0.1746	ns	0.8483
Ova/Dex vs. Ova/Amox	-0.1115	ns	0.8977
Ova/Dex vs. Ova/Amox/Dex	0.4852	ns	0.5765
Ova/Clari vs. Ova/Clari/Dex	2.331	*	0.0172
Ova/Clari vs. Ova/Amox	2.394	*	0.0107
Ova/Clari vs. Ova/Amox/Dex	2.991	**	0.0018
Ova/Clari/Dex vs. Ova/Amox	0.06309	ns	0.9467
Ova/Clari/Dex vs. Ova/Amox/Dex	0.6599	ns	0.4857
Ova/Amox vs. Ova/Amox/Dex	0.5968	ns	0.5083

D

One way ANOVA (Macrophages)	Mean Diff.	Summary	P Value
Sal vs. Ova	-11.2	****	< 0.0001
Sal vs. Ova/Dex	-3.736	*	0.0291
Sal vs. Ova/Clari	-7.779	****	< 0.0001
Sal vs. Ova/Clari/Dex	-9.316	****	< 0.0001
Sal vs. Ova/Amox	-18.71	****	< 0.0001
Sal vs. Ova/Amox/Dex	-3.038	ns	0.0858
Ova vs. Ova/Dex	7.468	***	0.0002
Ova vs. Ova/Clari	3.425	ns	0.0774
Ova vs. Ova/Clari/Dex	1.888	ns	0.3462
Ova vs. Ova/Amox	-7.504	**	0.0058
Ova vs. Ova/Amox/Dex	8.166	***	0.0001
Ova/Dex vs. Ova/Clari	-4.043	*	0.0322
Ova/Dex vs. Ova/Clari/Dex	-5.581	**	0.0059
Ova/Dex vs. Ova/Amox	-14.97	****	< 0.0001
Ova/Dex vs. Ova/Amox/Dex	0.6974	ns	0.7034
Ova/Clari vs. Ova/Clari/Dex	-1.537	ns	0.4421
Ova/Clari vs. Ova/Amox	-10.93	****	< 0.0001
Ova/Clari vs. Ova/Amox/Dex	4.741	*	0.0164
Ova/Clari/Dex vs. Ova/Amox	-9.391	****	< 0.0001
Ova/Clari/Dex vs. Ova/Amox/Dex	6.278	**	0.003
Ova/Amox vs. Ova/Amox/Dex	15.67	****	< 0.0001

Supplementary Figure 2: Bronchoalveolar lavage fluid (BALF) full statistical analyses for all steroid-sensitive allergic airways disease (AAD) groups.

A

One way ANOVA (Total cells)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-36.72	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Dex	-42.94	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Clari	-18.2	**	0.0053
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-16.81	**	0.0095
Sal/Cmu vs. Ova/Cmu/Amox	-26.15	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Amox/Dex	-3.443	ns	0.5581
Ova/Cmu vs. Ova/Cmu/Dex	-6.224	ns	0.2874
Ova/Cmu vs. Ova/Cmu/Clari	18.52	**	0.0058
Ova/Cmu vs. Ova/Cmu/Clari/Dex	19.91	**	0.0032
Ova/Cmu vs. Ova/Cmu/Amox	10.57	ns	0.0865
Ova/Cmu vs. Ova/Cmu/Amox/Dex	33.28	****	< 0.0001
Ova/Cmu/Dex vs. Ova/Cmu/Clari	24.74	***	0.0004
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	26.13	***	0.0002
Ova/Cmu/Dex vs. Ova/Cmu/Amox	16.79	**	0.0081
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	39.5	****	< 0.0001
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	1.391	ns	0.8396
Ova/Cmu/Clari vs. Ova/Cmu/Amox	-7.947	ns	0.2314
Ova/Cmu/Clari vs. Ova/Cmu/Amox/Dex	14.76	*	0.0298
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox	-9.338	ns	0.1612
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox/Dex	13.37	*	0.0478
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	22.71	***	0.0008

B

One way ANOVA (Neutrophils)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-15.84	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Dex	-20.39	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Clari	-8.185	*	0.0185
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-14.08	***	0.0001
Sal/Cmu vs. Ova/Cmu/Amox	-4.783	ns	0.1368
Sal/Cmu vs. Ova/Cmu/Amox/Dex	-3.688	ns	0.2484
Ova/Cmu vs. Ova/Cmu/Dex	-4.555	ns	0.1681
Ova/Cmu vs. Ova/Cmu/Clari	7.651	*	0.031
Ova/Cmu vs. Ova/Cmu/Clari/Dex	1.759	ns	0.6089
Ova/Cmu vs. Ova/Cmu/Amox	11.05	**	0.0016
Ova/Cmu vs. Ova/Cmu/Amox/Dex	12.15	***	0.0006
Ova/Cmu/Dex vs. Ova/Cmu/Clari	12.21	**	0.0014
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	6.314	ns	0.0816
Ova/Cmu/Dex vs. Ova/Cmu/Amox	15.61	****	< 0.0001
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	16.7	****	< 0.0001
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	-5.892	ns	0.1182
Ova/Cmu/Clari vs. Ova/Cmu/Amox	3.402	ns	0.3408
Ova/Cmu/Clari vs. Ova/Cmu/Amox/Dex	4.497	ns	0.2101
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox	9.294	*	0.0123
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox/Dex	10.39	**	0.0056
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	1.095	ns	0.7463

C

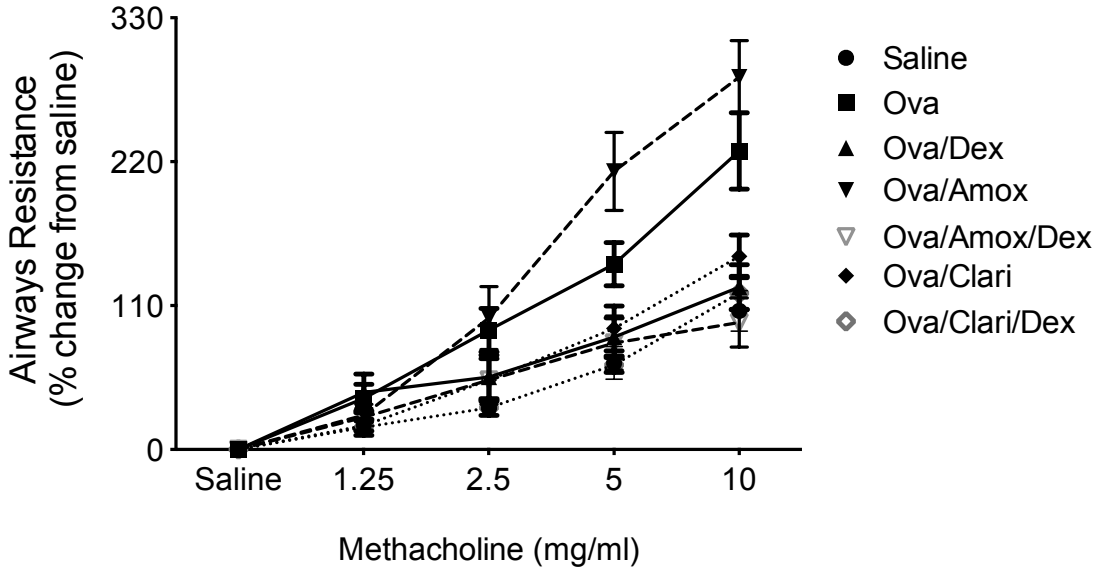
One way ANOVA (Eosinophils)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-1.437	***	0.0005
Sal/Cmu vs. Ova/Cmu/Dex	-0.9842	*	0.0166
Sal/Cmu vs. Ova/Cmu/Clari	-1.87	***	0.0001
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-0.6603	ns	0.1353
Sal/Cmu vs. Ova/Cmu/Amox	-0.2568	ns	0.5348
Sal/Cmu vs. Ova/Cmu/Amox/Dex	-0.1702	ns	0.6803
Ova/Cmu vs. Ova/Cmu/Dex	0.453	ns	0.256
Ova/Cmu vs. Ova/Cmu/Clari	-0.4331	ns	0.3232
Ova/Cmu vs. Ova/Cmu/Clari/Dex	0.777	ns	0.0805
Ova/Cmu vs. Ova/Cmu/Amox	1.18	**	0.0065
Ova/Cmu vs. Ova/Cmu/Amox/Dex	1.267	**	0.0037
Ova/Cmu/Dex vs. Ova/Cmu/Clari	-0.8861	ns	0.0534
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	0.3239	ns	0.4706
Ova/Cmu/Dex vs. Ova/Cmu/Amox	0.7274	ns	0.0931
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	0.814	ns	0.0614
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	1.21	*	0.016
Ova/Cmu/Clari vs. Ova/Cmu/Amox	1.614	**	0.0012
Ova/Cmu/Clari vs. Ova/Cmu/Amox/Dex	1.7	***	0.0007
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox	0.4035	ns	0.3855
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox/Dex	0.4901	ns	0.293
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	0.08658	ns	0.8444

D

One way ANOVA (Macrophages)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-16.4	***	0.0001
Sal/Cmu vs. Ova/Cmu/Dex	-12.37	**	0.0012
Sal/Cmu vs. Ova/Cmu/Clari	-6.777	ns	0.0897
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-1.073	ns	0.7986
Sal/Cmu vs. Ova/Cmu/Amox	-20.12	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Amox/Dex	0.4851	ns	0.8959
Ova/Cmu vs. Ova/Cmu/Dex	4.023	ns	0.2769
Ova/Cmu vs. Ova/Cmu/Clari	9.619	*	0.0212
Ova/Cmu vs. Ova/Cmu/Clari/Dex	15.32	***	0.001
Ova/Cmu vs. Ova/Cmu/Amox	-3.72	ns	0.3332
Ova/Cmu vs. Ova/Cmu/Amox/Dex	16.88	****	< 0.0001
Ova/Cmu/Dex vs. Ova/Cmu/Clari	5.596	ns	0.1695
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	11.3	*	0.012
Ova/Cmu/Dex vs. Ova/Cmu/Amox	-7.743	*	0.0486
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	12.86	**	0.0017
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	5.704	ns	0.2203
Ova/Cmu/Clari vs. Ova/Cmu/Amox	-13.34	**	0.0026
Ova/Cmu/Clari vs. Ova/Cmu/Amox/Dex	7.262	ns	0.087
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox	-19.04	***	0.0001
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox/Dex	1.558	ns	0.7254
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	20.6	****	< 0.0001

Supplementary Figure 3: BALF full statistical analyses for all severe, steroid-insensitive AAD groups (SSIAAD).

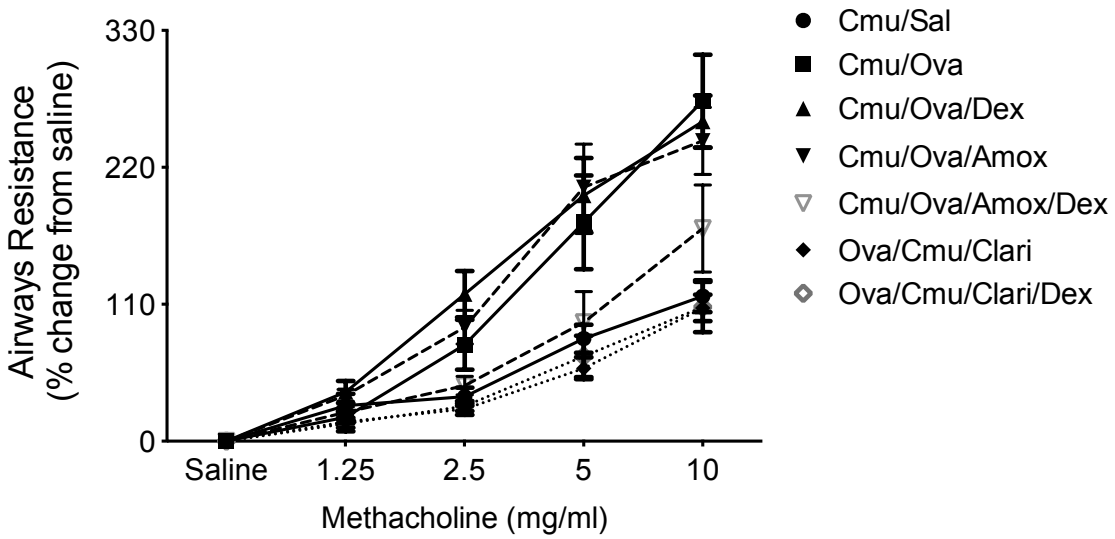
A



B

One way ANOVA (10mg/ml Mch)	Mean Diff.	Summary	P Value
Sal vs. Ova	-122.3	***	0.0001
Sal vs. Ova/Dex	-18.22	ns	0.5587
Sal vs. Ova/Clari	-41.77	ns	0.1471
Sal vs. Ova/Clari/Dex	-13.66	ns	0.6734
Sal vs. Ova/Amox	-178.7	****	< 0.0001
Sal vs. Ova/Amox/Dex	8.873	ns	0.7682
Ova vs. Ova/Dex	104.1	**	0.0017
Ova vs. Ova/Clari	80.51	**	0.0076
Ova vs. Ova/Clari/Dex	108.6	**	0.0016
Ova vs. Ova/Amox	-56.46	ns	0.0573
Ova vs. Ova/Amox/Dex	131.2	***	0.0001
Ova/Dex vs. Ova/Clari	-23.55	ns	0.4503
Ova/Dex vs. Ova/Clari/Dex	4.564	ns	0.8952
Ova/Dex vs. Ova/Amox	-160.5	****	< 0.0001
Ova/Dex vs. Ova/Amox/Dex	27.09	ns	0.4064
Ova/Clari vs. Ova/Clari/Dex	28.11	ns	0.3867
Ova/Clari vs. Ova/Amox	-137	****	< 0.0001
Ova/Clari vs. Ova/Amox/Dex	50.64	ns	0.0963
Ova/Clari/Dex vs. Ova/Amox	-165.1	****	< 0.0001
Ova/Clari/Dex vs. Ova/Amox/Dex	22.53	ns	0.5052
Ova/Amox vs. Ova/Amox/Dex	187.6	****	< 0.0001

C



D

One Way ANOVA (10mg/ml Mch)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-156.7	***	0.0001
Sal/Cmu vs. Ova/Cmu/Dex	-126.7	***	0.0007
Sal/Cmu vs. Ova/Cmu/Clari	9.503	ns	0.7934
Sal/Cmu vs. Ova/Cmu/Clari/Dex	8.657	ns	0.8114
Sal/Cmu vs. Ova/Cmu/Amox	-124.8	**	0.001
Sal/Cmu vs. Ova/Cmu/Amox/Dex	-42.36	ns	0.2599
Ova/Cmu vs. Ova/Cmu/Dex	30.05	ns	0.3841
Ova/Cmu vs. Ova/Cmu/Clari	166.2	***	0.0001
Ova/Cmu vs. Ova/Cmu/Clari/Dex	165.4	***	0.0001
Ova/Cmu vs. Ova/Cmu/Amox	31.92	ns	0.3684
Ova/Cmu vs. Ova/Cmu/Amox/Dex	114.4	**	0.0026
Ova/Cmu/Dex vs. Ova/Cmu/Clari	136.2	***	0.0003
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	135.3	***	0.0003
Ova/Cmu/Dex vs. Ova/Cmu/Amox	1.867	ns	0.9579
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	84.31	*	0.0239
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	-0.8453	ns	0.9814
Ova/Cmu/Clari vs. Ova/Cmu/Amox	-134.3	***	0.0005
Ova/Cmu/Clari vs. Ova/Cmu/Amox/Dex	-51.86	ns	0.169
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox	-133.5	***	0.0005
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox/Dex	-51.02	ns	0.1759
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	82.45	*	0.0308

Supplementary Figure 4: Clarithromycin and not amoxicillin suppresses airways hyperresponsiveness (AHR) in both steroid-sensitive and SSI AAD groups.

A

One Way ANOVA (LN IL-5)	Mean Diff.	Summary	P Value
Sal vs. Ova	-16.5	***	0.0001
Sal vs. Ova/Dex	-6.815	ns	0.1059
Sal vs. Ova/Amox	-13.68	**	0.002
Sal vs. Ova/Amox/Dex	-6.887	ns	0.0802
Sal vs. Ova/Clari	-4.893	ns	0.2594
Sal vs. Ova/Clari/Dex	-6.145	ns	0.2723
Ova vs. Ova/Dex	9.682	**	0.0057
Ova vs. Ova/Amox	2.818	ns	0.3968
Ova vs. Ova/Amox/Dex	9.61	**	0.0023
Ova vs. Ova/Clari	11.6	**	0.0021
Ova vs. Ova/Clari/Dex	10.35	*	0.0429
Ova/Dex vs. Ova/Amox	-6.864	ns	0.07
Ova/Dex vs. Ova/Amox/Dex	-0.07223	ns	0.9829
Ova/Dex vs. Ova/Clari	1.922	ns	0.6209
Ova/Dex vs. Ova/Clari/Dex	0.6695	ns	0.8982
Ova/Amox vs. Ova/Amox/Dex	6.792	ns	0.0505
Ova/Amox vs. Ova/Clari	8.786	*	0.0288
Ova/Amox vs. Ova/Clari/Dex	7.533	ns	0.1559
Ova/Amox/Dex vs. Ova/Clari	1.994	ns	0.5777
Ova/Amox/Dex vs. Ova/Clari/Dex	0.7417	ns	0.8823
Ova/Clari vs. Ova/Clari/Dex	-1.253	ns	0.8153

B

One Way ANOVA (LN IL-13)	Mean Diff.	Summary	P Value
Sal vs. Ova	-18.43	****	< 0.0001
Sal vs. Ova/Dex	4.385	ns	0.2444
Sal vs. Ova/Amox	2.256	ns	0.5274
Sal vs. Ova/Amox/Dex	2.5	ns	0.4962
Sal vs. Ova/Clari	3.65	ns	0.3994
Sal vs. Ova/Clar/Dex	-0.117	ns	0.98
Ova vs. Ova/Dex	22.82	****	< 0.0001
Ova vs. Ova/Amox	20.69	****	< 0.0001
Ova vs. Ova/Amox/Dex	20.93	****	< 0.0001
Ova vs. Ova/Clari	22.08	****	< 0.0001
Ova vs. Ova/Clar/Dex	18.31	***	0.0001
Ova/Dex vs. Ova/Amox	-2.129	ns	0.454
Ova/Dex vs. Ova/Amox/Dex	-1.884	ns	0.5258
Ova/Dex vs. Ova/Clari	-0.735	ns	0.844
Ova/Dex vs. Ova/Clar/Dex	-4.502	ns	0.2791
Ova/Amox vs. Ova/Amox/Dex	0.2446	ns	0.9289
Ova/Amox vs. Ova/Clari	1.394	ns	0.6956
Ova/Amox vs. Ova/Clar/Dex	-2.373	ns	0.5511
Ova/Amox/Dex vs. Ova/Clari	1.149	ns	0.7539
Ova/Amox/Dex vs. Ova/Clar/Dex	-2.617	ns	0.5207
Ova/Clari vs. Ova/Clar/Dex	-3.767	ns	0.4205

C

One Way ANOVA (LN TNFα)	Mean Diff.	Summary	P Value
Sal vs. Ova	-245	*	0.0264
Sal vs. Ova/Dex	-109.8	ns	0.3468
Sal vs. Ova/Amox	-271.7	**	0.0092
Sal vs. Ova/Amox/Dex	-2.561	ns	0.9807
Sal vs. Ova/Clari	36.31	ns	0.7541
Sal vs. Ova/Clari/Dex	15.23	ns	0.8954
Ova vs. Ova/Dex	135.2	ns	0.2073
Ova vs. Ova/Amox	-26.75	ns	0.7569
Ova vs. Ova/Amox/Dex	242.4	*	0.0149
Ova vs. Ova/Clari	281.3	*	0.0118
Ova vs. Ova/Clari/Dex	260.2	*	0.0189
Ova/Dex vs. Ova/Amox	-161.9	ns	0.1076
Ova/Dex vs. Ova/Amox/Dex	107.2	ns	0.3147
Ova/Dex vs. Ova/Clari	146.1	ns	0.2132
Ova/Dex vs. Ova/Clari/Dex	125	ns	0.2851
Ova/Amox vs. Ova/Amox/Dex	269.1	**	0.0037
Ova/Amox vs. Ova/Clari	308	**	0.0036
Ova/Amox vs. Ova/Clari/Dex	286.9	**	0.0063
Ova/Amox/Dex vs. Ova/Clari	38.88	ns	0.7134
Ova/Amox/Dex vs. Ova/Clari/Dex	17.79	ns	0.8664
Ova/Clari vs. Ova/Clari/Dex	-21.08	ns	0.8556

D

One Way ANOVA (LN IL-5)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-6.628	*	0.0132
Sal/Cmu vs. Ova/Cmu/Dex	-8.195	***	0.0008
Sal/Cmu vs. Ova/Cmu/Amox	-7.154	**	0.0019
Sal/Cmu vs. Ova/Cmu/Amox/Dex	-4.672	*	0.0411
Sal/Cmu vs. Ova/Cmu/Clari	-7.497	**	0.0085
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-4.909	ns	0.0778
Ova/Cmu vs. Ova/Cmu/Dex	-1.566	ns	0.4916
Ova/Cmu vs. Ova/Cmu/Amox	-0.5256	ns	0.8085
Ova/Cmu vs. Ova/Cmu/Amox/Dex	1.956	ns	0.3826
Ova/Cmu vs. Ova/Cmu/Clari	-0.8691	ns	0.7505
Ova/Cmu vs. Ova/Cmu/Clari/Dex	1.72	ns	0.5299
Ova/Cmu/Dex vs. Ova/Cmu/Amox	1.041	ns	0.5629
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	3.522	ns	0.0651
Ova/Cmu/Dex vs. Ova/Cmu/Clari	0.6972	ns	0.7758
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	3.286	ns	0.1839
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	2.481	ns	0.1596
Ova/Cmu/Amox vs. Ova/Cmu/Clari	-0.3436	ns	0.8838
Ova/Cmu/Amox vs. Ova/Cmu/Clari/Dex	2.245	ns	0.3421
Ova/Cmu/Amox/Dex vs. Ova/Cmu/Clari	-2.825	ns	0.2447
Ova/Cmu/Amox/Dex vs. Ova/Cmu/Clari/Dex	-0.2363	ns	0.9219
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	2.589	ns	0.3709

E

One Way ANOVA (LN IL-13)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-4.361	ns	0.1342
Sal/Cmu vs. Ova/Cmu/Dex	-1.264	ns	0.6432
Sal/Cmu vs. Ova/Cmu/Amox	6.965	*	0.0151
Sal/Cmu vs. Ova/Cmu/Amox/Dex	7.519	**	0.0092
Sal/Cmu vs. Ova/Cmu/Clari	-2.037	ns	0.5051
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-5.944	ns	0.0584
Ova/Cmu vs. Ova/Cmu/Dex	3.097	ns	0.2831
Ova/Cmu vs. Ova/Cmu/Amox	11.33	**	0.004
Ova/Cmu vs. Ova/Cmu/Amox/Dex	11.88	**	0.002
Ova/Cmu vs. Ova/Cmu/Clari	2.324	ns	0.4649
Ova/Cmu vs. Ova/Cmu/Clari/Dex	-1.583	ns	0.6177
Ova/Cmu/Dex vs. Ova/Cmu/Amox	8.229	**	0.0048
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	8.783	**	0.0028
Ova/Cmu/Dex vs. Ova/Cmu/Clari	-0.7732	ns	0.7997
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	-4.68	ns	0.1318
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	0.5535	ns	0.839
Ova/Cmu/Amox vs. Ova/Cmu/Clari	-9.003	**	0.0057
Ova/Cmu/Amox vs. Ova/Cmu/Clari/Dex	-12.91	***	0.0002
Ova/Cmu/Amox/Dex vs. Ova/Cmu/Clari	-9.556	**	0.0036
Ova/Cmu/Amox/Dex vs. Ova/Cmu/Clari/Dex	-13.46	***	0.0001
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	-3.906	ns	0.247

F

One Way ANOVA (LN TNFα)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-391.6	*	0.0321
Sal/Cmu vs. Ova/Cmu/Dex	-270.4	ns	0.1566
Sal/Cmu vs. Ova/Cmu/Amox	-303.2	ns	0.0811
Sal/Cmu vs. Ova/Cmu/Amox/Dex	-271	ns	0.1322
Sal/Cmu vs. Ova/Cmu/Clari	-71.49	ns	0.7046
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-80.68	ns	0.7227
Ova/Cmu vs. Ova/Cmu/Dex	121.2	ns	0.472
Ova/Cmu vs. Ova/Cmu/Amox	88.41	ns	0.55
Ova/Cmu vs. Ova/Cmu/Amox/Dex	120.6	ns	0.4399
Ova/Cmu vs. Ova/Cmu/Clari	-123.3	*	0.0140
Ova/Cmu vs. Ova/Cmu/Clari/Dex	-145.3	*	0.0242
Ova/Cmu/Dex vs. Ova/Cmu/Amox	-32.82	ns	0.8382
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	-0.6147	ns	0.9971
Ova/Cmu/Dex vs. Ova/Cmu/Clari	198.9	ns	0.2719
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	189.7	ns	0.3908
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	32.21	ns	0.8273
Ova/Cmu/Amox vs. Ova/Cmu/Clari	231.7	ns	0.1546
Ova/Cmu/Amox vs. Ova/Cmu/Clari/Dex	222.5	ns	0.2809
Ova/Cmu/Amox/Dex vs. Ova/Cmu/Clari	199.5	ns	0.2393
Ova/Cmu/Amox/Dex vs. Ova/Cmu/Clari/Dex	190.3	ns	0.3687
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	-9.194	ns	0.9667

G

One Way ANOVA (Lung IL-5)	Mean Diff.	Summary	P Value
Sal vs. Ova	-0.004648	*	0.0118
Sal vs. Ova/Dex	-0.002653	ns	0.1046
Sal vs. Ova/Clari	-0.003961	*	0.0243
Sal vs. Ova/Clari/Dex	-0.003023	ns	0.0805
Sal vs. OVA/Amox	-0.006989	***	0.0006
Sal vs. Ova/Amox/Dex	-0.001523	ns	0.3878
Ova vs. Ova/Dex	0.001995	ns	0.1866
Ova vs. Ova/Clari	0.0006874	ns	0.6643
Ova vs. Ova/Clari/Dex	0.001626	ns	0.3082
Ova vs. OVA/Amox	-0.002341	ns	0.1878
Ova vs. Ova/Amox/Dex	-0.003126	*	0.0463
Ova/Dex vs. Ova/Clari	-0.001308	ns	0.3573
Ova/Dex vs. Ova/Clari/Dex	-0.0003694	ns	0.7936
Ova/Dex vs. OVA/Amox	-0.004336	*	0.0103
Ova/Dex vs. Ova/Amox/Dex	0.001131	ns	0.4498
Ova/Clari vs. Ova/Clari/Dex	0.0009382	ns	0.5352
Ova/Clari vs. OVA/Amox	-0.003028	ns	0.0799
Ova/Clari vs. Ova/Amox/Dex	0.002438	ns	0.1303
Ova/Clari/Dex vs. OVA/Amox	-0.003967	*	0.0241
Ova/Clari/Dex vs. Ova/Amox/Dex	0.0015	ns	0.3465
OVA/Amox vs. Ova/Amox/Dex	0.005467	**	0.0036

H

One Way ANOVA (Lung IL-13)	Mean Diff.	Summary	P Value
Sal vs. Ova	-0.0226	***	0.0005
Sal vs. Ova/Dex	-0.00405	ns	0.4897
Sal vs. Ova/Clari	-0.009566	ns	0.1095
Sal vs. Ova/Clari/Dex	-0.03294	****	< 0.0001
Sal vs. OVA/Amox	-0.02017	**	0.0036
Sal vs. Ova/Amox/Dex	-0.02254	***	0.0008
Ova vs. Ova/Dex	0.01855	**	0.0013
Ova vs. Ova/Clari	0.01304	*	0.0178
Ova vs. Ova/Clari/Dex	-0.01034	ns	0.0848
Ova vs. OVA/Amox	0.002427	ns	0.6782
Ova vs. Ova/Amox/Dex	6.13E-05	ns	0.9911
Ova/Dex vs. Ova/Clari	-0.005515	ns	0.2957
Ova/Dex vs. Ova/Clari/Dex	-0.02889	****	< 0.0001
Ova/Dex vs. OVA/Amox	-0.01612	**	0.0095
Ova/Dex vs. Ova/Amox/Dex	-0.01849	**	0.002
Ova/Clari vs. Ova/Clari/Dex	-0.02337	***	0.0004
Ova/Clari vs. OVA/Amox	-0.01061	ns	0.0774
Ova/Clari vs. Ova/Amox/Dex	-0.01297	*	0.0238
Ova/Clari/Dex vs. OVA/Amox	0.01277	ns	0.0537
Ova/Clari/Dex vs. Ova/Amox/Dex	0.0104	ns	0.0947
OVA/Amox vs. Ova/Amox/Dex	-0.002365	ns	0.6971

I

One Way ANOVA (Lung TNFα)	Mean Diff.	Summary	P Value
Sal vs. Ova	-0.04439	**	0.0022
Sal vs. Ova/Dex	-0.0131	ns	0.2905
Sal vs. Ova/Clari	-0.05271	***	0.0004
Sal vs. Ova/Clari/Dex	-0.01317	ns	0.3359
Sal vs. OVA/Amox	-0.06977	****	< 0.0001
Sal vs. Ova/Amox/Dex	-0.01383	ns	0.3123
Ova vs. Ova/Dex	0.03129	**	0.0055
Ova vs. Ova/Clari	-0.008316	ns	0.4955
Ova vs. Ova/Clari/Dex	0.03122	*	0.0138
Ova vs. OVA/Amox	-0.02538	ns	0.0525
Ova vs. Ova/Amox/Dex	0.03056	*	0.0158
Ova/Dex vs. Ova/Clari	-0.03961	***	0.0006
Ova/Dex vs. Ova/Clari/Dex	-6.69E-05	ns	0.995
Ova/Dex vs. OVA/Amox	-0.05667	****	< 0.0001
Ova/Dex vs. Ova/Amox/Dex	-0.0007336	ns	0.9453
Ova/Clari vs. Ova/Clari/Dex	0.03954	**	0.0023
Ova/Clari vs. OVA/Amox	-0.01707	ns	0.1861
Ova/Clari vs. Ova/Amox/Dex	0.03887	**	0.0027
Ova/Clari/Dex vs. OVA/Amox	-0.05661	****	< 0.0001
Ova/Clari/Dex vs. Ova/Amox/Dex	-0.0006667	ns	0.9563
OVA/Amox vs. Ova/Amox/Dex	0.05594	****	< 0.0001

J

One Way ANOVA (Lung IL-5)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-0.002613	*	0.042
Sal/Cmu vs. Ova/Cmu/Dex	-0.004932	***	0.0004
Sal/Cmu vs. Ova/Cmu/Clari	-0.002052	ns	0.1193
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-0.002638	*	0.0479
Sal/Cmu vs. Ova/Cmu/Amox	-0.009516	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Amox/Dex	-0.002338	ns	0.0672
Ova/Cmu vs. Ova/Cmu/Dex	-0.002319	*	0.0436
Ova/Cmu vs. Ova/Cmu/Clari	0.0005616	ns	0.6305
Ova/Cmu vs. Ova/Cmu/Clari/Dex	-2.42E-05	ns	0.9835
Ova/Cmu vs. Ova/Cmu/Amox	-0.006903	***	0.0001
Ova/Cmu vs. Ova/Cmu/Amox/Dex	0.0002758	ns	0.804
Ova/Cmu/Dex vs. Ova/Cmu/Clari	0.00288	*	0.0183
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	0.002295	ns	0.0561
Ova/Cmu/Dex vs. Ova/Cmu/Amox	-0.004584	***	0.0002
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	0.002595	*	0.0251
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	-0.0005858	ns	0.631
Ova/Cmu/Clari vs. Ova/Cmu/Amox	-0.007464	****	< 0.0001
Ova/Cmu/Clari vs. Ova/Cmu/Amox/Dex	-0.0002858	ns	0.8064
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox	-0.006879	****	< 0.0001
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox/Dex	0.0003	ns	0.7969
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	0.007179	****	< 0.0001

K

One Way ANOVA (Lung IL-13)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-0.008211	**	0.0053
Sal/Cmu vs. Ova/Cmu/Dex	-0.01093	***	0.0004
Sal/Cmu vs. Ova/Cmu/Clari	-0.005066	ns	0.0837
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-0.01958	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Amox	-0.02586	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Amox/Dex	-0.01913	****	< 0.0001
Ova/Cmu vs. Ova/Cmu/Dex	-0.002716	ns	0.2737
Ova/Cmu vs. Ova/Cmu/Clari	0.003144	ns	0.2279
Ova/Cmu vs. Ova/Cmu/Clari/Dex	-0.01136	***	0.0002
Ova/Cmu vs. Ova/Cmu/Amox	-0.01765	****	< 0.0001
Ova/Cmu vs. Ova/Cmu/Amox/Dex	-0.01091	***	0.0002
Ova/Cmu/Dex vs. Ova/Cmu/Clari	0.00586	*	0.0291
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	-0.008649	**	0.0035
Ova/Cmu/Dex vs. Ova/Cmu/Amox	-0.01494	****	< 0.0001
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	-0.008199	**	0.0032
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	-0.01451	****	< 0.0001
Ova/Cmu/Clari vs. Ova/Cmu/Amox	-0.0208	****	< 0.0001
Ova/Cmu/Clari vs. Ova/Cmu/Amox/Dex	-0.01406	****	< 0.0001
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox	-0.006289	*	0.0281
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox/Dex	0.00045	ns	0.8747
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	0.006739	*	0.0132

L

One Way ANOVA (Lung TNFα)	Mean Diff.	Summary	P Value
Sal/Cmu vs. Ova/Cmu	-0.2815	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Dex	-0.2116	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Clari	-0.07327	ns	0.0921
Sal/Cmu vs. Ova/Cmu/Clari/Dex	-0.09153	*	0.0378
Sal/Cmu vs. Ova/Cmu/Amox	-0.1945	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Amox/Dex	-0.0235	ns	0.5628
Ova/Cmu vs. Ova/Cmu/Dex	0.06994	ns	0.1223
Ova/Cmu vs. Ova/Cmu/Clari	0.2083	****	< 0.0001
Ova/Cmu vs. Ova/Cmu/Clari/Dex	0.19	***	0.0002
Ova/Cmu vs. Ova/Cmu/Amox	0.08703	ns	0.0571
Ova/Cmu vs. Ova/Cmu/Amox/Dex	0.258	****	< 0.0001
Ova/Cmu/Dex vs. Ova/Cmu/Clari	0.1383	**	0.0037
Ova/Cmu/Dex vs. Ova/Cmu/Clari/Dex	0.1201	*	0.0105
Ova/Cmu/Dex vs. Ova/Cmu/Amox	0.01709	ns	0.7005
Ova/Cmu/Dex vs. Ova/Cmu/Amox/Dex	0.1881	***	0.0001
Ova/Cmu/Clari vs. Ova/Cmu/Clari/Dex	-0.01826	ns	0.681
Ova/Cmu/Clari vs. Ova/Cmu/Amox	-0.1212	**	0.0098
Ova/Cmu/Clari vs. Ova/Cmu/Amox/Dex	0.04977	ns	0.2466
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox	-0.103	*	0.0261
Ova/Cmu/Clari/Dex vs. Ova/Cmu/Amox/Dex	0.06803	ns	0.1167
Ova/Cmu/Amox vs. Ova/Cmu/Amox/Dex	0.171	***	0.0003

Supplementary Figure 5: Inflammatory mediator full statistical analyses for all steroid-sensitive and SSIAAD groups.

A

One way ANOVA (Total cells)	Mean Diff.	Summary	P Value
Sal vs. Ova	-25.3	***	0.0001
Sal vs. Ova/Dex	-0.7806	ns	0.8914
Sal vs. Sal/Cmu	-14.54	*	0.0352
Sal vs. Ova/Cmu	-51.13	****	< 0.0001
Sal vs. Ova/Cmu/Dex	-41.47	****	< 0.0001
Sal vs. Ova/Cmu/αTNF-α	-13.62	*	0.0309
Ova vs. Ova/Dex	24.52	***	0.0001
Ova vs. Sal/Cmu	10.75	ns	0.058
Ova vs. Ova/Cmu	-25.84	***	0.0001
Ova vs. Ova/Cmu/Dex	-16.18	**	0.0028
Ova vs. Ova/Cmu/αTNF-α	11.67	*	0.0192
Ova vs. Ova/Cmu/αTNF-α/Dex	26	****	< 0.0001
Ova/Dex vs. Sal/Cmu	-13.76	*	0.0197
Ova/Dex vs. Ova/Cmu	-50.35	****	< 0.0001
Ova/Dex vs. Ova/Cmu/Dex	-40.69	****	< 0.0001
Ova/Dex vs. Ova/Cmu/αTNF-α	-12.84	*	0.0135
Sal/Cmu vs. Ova/Cmu	-36.59	***	0.0001
Sal/Cmu vs. Ova/Cmu/Dex	-26.93	***	0.0001
Sal/Cmu vs. Ova/Cmu/αTNF-α	0.9192	ns	0.881
Ova/Cmu vs. Ova/Cmu/Dex	9.658	ns	0.0989
Ova/Cmu vs. Ova/Cmu/αTNF-α	37.51	***	0.0001
Ova/Cmu/Dex vs. Ova/Cmu/αTNF-α	27.85	***	0.0001

B

One way ANOVA (Neutrophils)	Mean Diff.	Summary	P Value
Sal vs. Ova	2.648	*	0.05
Sal vs. Ova/Dex	-1.942	ns	0.4631
Sal vs. Sal/Cmu	-2.153	ns	0.4891
Sal vs. Ova/Cmu	-19.67	****	< 0.0001
Sal vs. Ova/Cmu/Dex	-17.98	****	< 0.0001
Sal vs. Ova/Cmu/αTNF-α	-4.28	ns	0.1512
Ova vs. Ova/Dex	0.7065	ns	0.7205
Ova vs. Sal/Cmu	0.495	ns	0.8469
Ova vs. Ova/Cmu	-17.03	***	0.0001
Ova vs. Ova/Cmu/Dex	-15.33	****	< 0.0001
Ova vs. Ova/Cmu/αTNF-α	-1.631	ns	0.492
Ova/Dex vs. Sal/Cmu	-0.2115	ns	0.9361
Ova/Dex vs. Ova/Cmu	-17.73	****	< 0.0001
Ova/Dex vs. Ova/Cmu/Dex	-16.03	****	< 0.0001
Ova/Dex vs. Ova/Cmu/αTNF-α	-2.338	ns	0.3423
Sal/Cmu vs. Ova/Cmu	-17.52	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Dex	-15.82	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/αTNF-α	-2.126	ns	0.4716
Ova/Cmu vs. Ova/Cmu/Dex	1.698	ns	0.5239
Ova/Cmu vs. Ova/Cmu/αTNF-α	15.39	****	< 0.0001
Ova/Cmu/Dex vs. Ova/Cmu/αTNF-α	13.7	***	0.0001

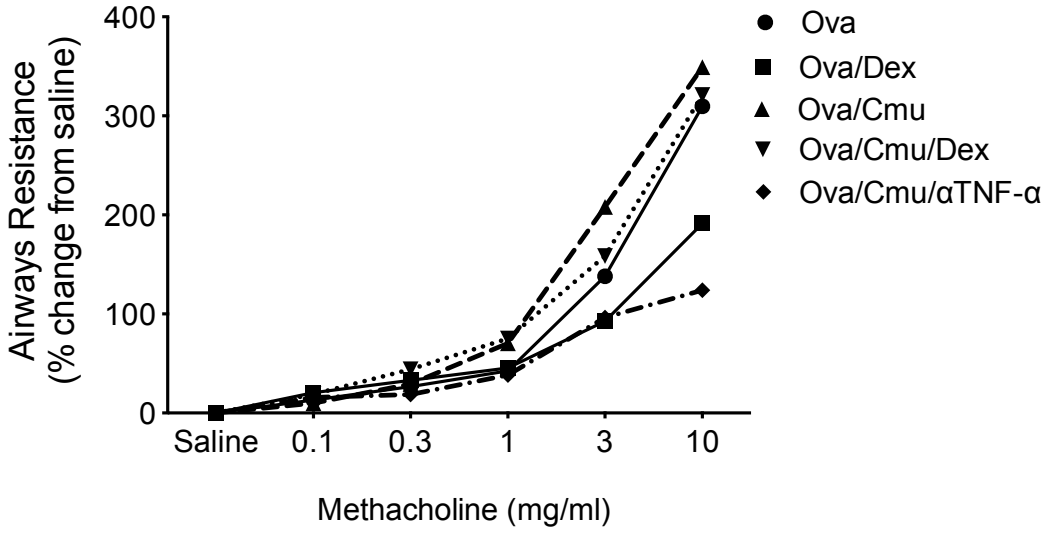
C

One way ANOVA (Eosinophils)	Mean Diff.	Summary	P Value
Sal vs. Ova	-7.828	****	< 0.0001
Sal vs. Ova/Dex	-0.426	ns	0.6408
Sal vs. Sal/Cmu	0.06313	ns	0.9531
Sal vs. Ova/Cmu	-4.763	****	< 0.0001
Sal vs. Ova/Cmu/Dex	-2.841	**	0.0076
Sal vs. Ova/Cmu/αTNF-α	-1.918	ns	0.0651
Ova vs. Ova/Dex	7.402	***	0.0001
Ova vs. Sal/Cmu	7.891	****	< 0.0001
Ova vs. Ova/Cmu	3.065	**	0.005
Ova vs. Ova/Cmu/Dex	4.987	****	< 0.0001
Ova vs. Ova/Cmu/αTNF-α	5.911	****	< 0.0001
Ova/Dex vs. Sal/Cmu	0.4891	ns	0.5923
Ova/Dex vs. Ova/Cmu	-4.337	****	< 0.0001
Ova/Dex vs. Ova/Cmu/Dex	-2.415	**	0.0064
Ova/Dex vs. Ova/Cmu/αTNF-α	-1.492	ns	0.0836
Sal/Cmu vs. Ova/Cmu	-4.826	****	< 0.0001
Sal/Cmu vs. Ova/Cmu/Dex	-2.904	**	0.0064
Sal/Cmu vs. Ova/Cmu/αTNF-α	-1.981	ns	0.0571
Ova/Cmu vs. Ova/Cmu/Dex	1.922	ns	0.0505
Ova/Cmu vs. Ova/Cmu/αTNF-α	2.846	**	0.0048
Ova/Cmu/Dex vs. Ova/Cmu/αTNF-α	0.9234	ns	0.3386

D

One way ANOVA (Macrophages)	Mean Diff.	Summary	P Value
Sal vs. Ova	-11.99	***	0.0003
Sal vs. Ova/Dex	0.1099	ns	0.968
Sal vs. Sal/Cmu	-7.609	*	0.0162
Sal vs. Ova/Cmu	-19.54	****	< 0.0001
Sal vs. Ova/Cmu/Dex	-16.52	****	< 0.0001
Sal vs. Ova/Cmu/αTNF-α	-4.201	ns	0.1584
Ova vs. Ova/Dex	12.1	***	0.0005
Ova vs. Sal/Cmu	4.378	ns	0.0805
Ova vs. Ova/Cmu	-7.556	**	0.002
Ova vs. Ova/Cmu/Dex	-4.532	ns	0.0707
Ova vs. Ova/Cmu/αTNF-α	7.786	**	0.0015
Ova/Dex vs. Sal/Cmu	-7.719	**	0.0039
Ova/Dex vs. Ova/Cmu	-19.65	****	< 0.0001
Ova/Dex vs. Ova/Cmu/Dex	-16.63	****	< 0.0001
Ova/Dex vs. Ova/Cmu/αTNF-α	-4.311	ns	0.0782
Sal/Cmu vs. Ova/Cmu	-11.93	***	0.0001
Sal/Cmu vs. Ova/Cmu/Dex	-8.91	**	0.0033
Sal/Cmu vs. Ova/Cmu/αTNF-α	3.408	ns	0.2213
Ova/Cmu vs. Ova/Cmu/Dex	3.024	ns	0.2769
Ova/Cmu vs. Ova/Cmu/αTNF-α	15.34	***	0.0003
Ova/Cmu/Dex vs. Ova/Cmu/αTNF-α	12.32	***	0.0001

E



F

One Way ANOVA (10mg/ml Mch)	Mean Diff.	Summary	P Value
Sal vs. Ova	-134.8	**	0.009
Sal vs. Ova/Dex	1.699	ns	0.9725
Sal vs. Sal/Cmu	2.656	ns	0.9627
Sal vs. Ova/Cmu	-156.1	**	0.0045
Sal vs. Ova/Cmu/Dex	-128.1	*	0.0292
Sal vs. Ova/Cmu/αTNF-α	68.93	ns	0.2061
Ova vs. Ova/Dex	136.5	**	0.0015
Ova vs. Sal/Cmu	137.5	**	0.0078
Ova vs. Ova/Cmu	-21.32	ns	0.624
Ova vs. Ova/Cmu/Dex	6.75	ns	0.8909
Ova vs. Ova/Cmu/αTNF-α	203.7	****	< 0.0001
Ova/Dex vs. Sal/Cmu	0.9573	ns	0.9845
Ova/Dex vs. Ova/Cmu	-157.8	***	0.0008
Ova/Dex vs. Ova/Cmu/Dex	-129.8	*	0.0117
Ova/Dex vs. Ova/Cmu/αTNF-α	67.24	ns	0.1481
Sal/Cmu vs. Ova/Cmu	-158.8	**	0.0039
Sal/Cmu vs. Ova/Cmu/Dex	-130.7	*	0.0263
Sal/Cmu vs. Ova/Cmu/αTNF-α	66.28	ns	0.2237
Ova/Cmu vs. Ova/Cmu/Dex	28.07	ns	0.5893
Ova/Cmu vs. Ova/Cmu/αTNF-α	225.1	****	< 0.0001
Ova/Cmu/Dex vs. Ova/Cmu/αTNF-α	197	***	0.0007

Supplementary Figure 6: Anti-TNF-α therapy suppresses *Chlamydia*-induced SSIAAD.

A

One way ANOVA (Total cells)	Mean Diff.	Summary	P Value
Sal vs. Ova	-64.9	***	0.0001
Sal vs. Clari/Ova	-19	ns	0.148
Sal vs. Clari/Ova/Dex	-14.54	ns	0.2643
Sal vs. Hi/Sal	28.85	*	0.0198
Sal vs. Hi/Ova	-42	**	0.0028
Sal vs. Hi/Clari/Ova	-50.64	***	0.0003
Sal vs. Hi/Clari/Ova/Dex	-12.12	ns	0.3869
Ova vs. Clari/Ova	45.9	***	0.0008
Ova vs. Clari/Ova/Dex	50.36	***	0.0003
Ova vs. Hi/Sal	93.75	****	< 0.0001
Ova vs. Hi/Ova	22.9	ns	0.0692
Ova vs. Hi/Clari/Ova	14.26	ns	0.222
Ova vs. Hi/Clari/Ova/Dex	52.78	***	0.0004
Clari/Ova vs. Clari/Ova/Dex	4.458	ns	0.7294
Clari/Ova vs. Hi/Sal	47.85	***	0.0003
Clari/Ova vs. Hi/Ova	-23	ns	0.0826
Clari/Ova vs. Hi/Clari/Ova	-31.64	*	0.0144
Clari/Ova vs. Hi/Clari/Ova/Dex	6.881	ns	0.6216
Clari/Ova/Dex vs. Hi/Sal	43.39	***	0.0009
Clari/Ova/Dex vs. Hi/Ova	-27.46	*	0.0405
Clari/Ova/Dex vs. Hi/Clari/Ova	-36.1	**	0.006
Clari/Ova/Dex vs. Hi/Clari/Ova/Dex	2.423	ns	0.8617
Hi/Sal vs. Hi/Ova	-70.85	***	0.0003
Hi/Sal vs. Hi/Clari/Ova	-79.48	****	< 0.0001
Hi/Sal vs. Hi/Clari/Ova/Dex	-40.96	**	0.0034
Hi/Ova vs. Hi/Clari/Ova	-8.638	ns	0.4815
Hi/Ova vs. Hi/Clari/Ova/Dex	29.88	*	0.0391
Hi/Clari/Ova vs. Hi/Clari/Ova/Dex	38.52	**	0.0069

B

One way ANOVA (Neutrophils)	Mean Diff.	Summary	P Value
Sal vs. Ova	-4.306	***	0.0003
Sal vs. Clari/Ova	-1.37	ns	0.1886
Sal vs. Clari/Ova/Dex	-1.492	ns	0.1534
Sal vs. Hi/Sal	-1.05	ns	0.2849
Sal vs. Hi/Ova	-8.28	****	< 0.0001
Sal vs. Hi/Clari/Ova	-3.478	**	0.0021
Sal vs. Hi/Clari/Ova/Dex	-0.9572	ns	0.3539
Ova vs. Clari/Ova	2.937	**	0.0077
Ova vs. Clari/Ova/Dex	2.814	*	0.0102
Ova vs. Hi/Sal	3.256	**	0.0023
Ova vs. Hi/Ova	-3.974	***	0.0006
Ova vs. Hi/Clari/Ova	0.8277	ns	0.4217
Ova vs. Hi/Clari/Ova/Dex	3.349	**	0.0029
Clari/Ova vs. Clari/Ova/Dex	-0.1224	ns	0.9048
Clari/Ova vs. Hi/Sal	0.3191	ns	0.7427
Clari/Ova vs. Hi/Ova	-6.91	****	< 0.0001
Clari/Ova vs. Hi/Clari/Ova	-2.109	*	0.0478
Clari/Ova vs. Hi/Clari/Ova/Dex	0.4123	ns	0.6875
Clari/Ova/Dex vs. Hi/Sal	0.4415	ns	0.65
Clari/Ova/Dex vs. Hi/Ova	-6.788	****	< 0.0001
Clari/Ova/Dex vs. Hi/Clari/Ova	-1.987	ns	0.0612
Clari/Ova/Dex vs. Hi/Clari/Ova/Dex	0.5347	ns	0.6024
Hi/Sal vs. Hi/Ova	-7.23	***	0.0001
Hi/Sal vs. Hi/Clari/Ova	-2.428	*	0.0182
Hi/Sal vs. Hi/Clari/Ova/Dex	0.09319	ns	0.9235
Hi/Ova vs. Hi/Clari/Ova	4.802	**	0.0049
Hi/Ova vs. Hi/Clari/Ova/Dex	7.323	***	0.0008
Hi/Clari/Ova vs. Hi/Clari/Ova/Dex	2.521	*	0.0199

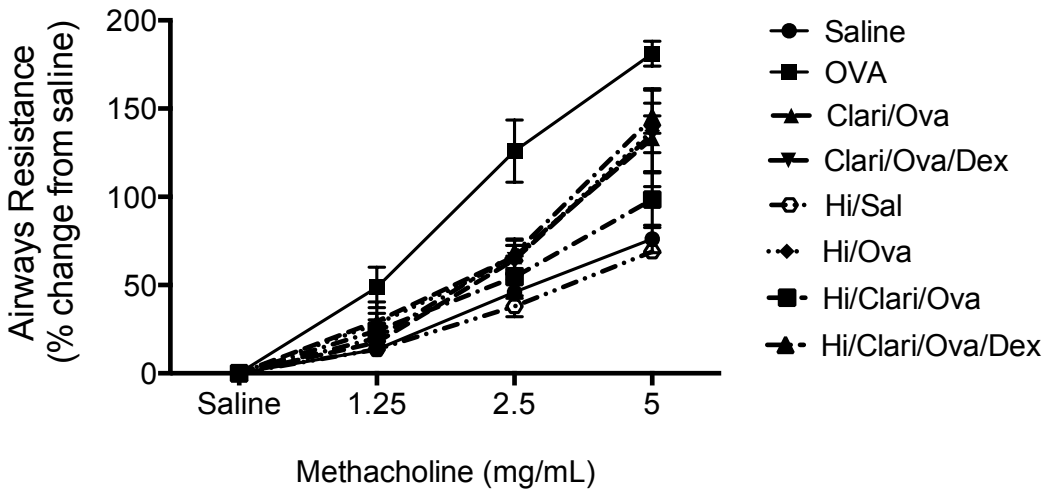
C

One way ANOVA (Eosinophils)	Mean Diff.	Summary	P Value
Sal vs. Ova	-6.207	****	< 0.0001
Sal vs. Clari/Ova	-1.922	**	0.0024
Sal vs. Clari/Ova/Dex	-2.418	***	0.0007
Sal vs. Hi/Sal	-0.1307	ns	0.8085
Sal vs. Hi/Ova	-3.327	****	< 0.0001
Sal vs. Hi/Clar/Ova	-5.3	****	< 0.0001
Sal vs. Hi/Clar/Ova/Dex	-0.7511	ns	0.2392
Ova vs. Clari/Ova	4.284	***	0.0004
Ova vs. Clari/Ova/Dex	3.788	***	0.0003
Ova vs. Hi/Sal	6.076	****	< 0.0001
Ova vs. Hi/Ova	2.879	**	0.005
Ova vs. Hi/Clar/Ova	0.9066	ns	0.158
Ova vs. Hi/Clar/Ova/Dex	5.455	****	< 0.0001
Clari/Ova vs. Clari/Ova/Dex	-0.4961	ns	0.4677
Clari/Ova vs. Hi/Sal	1.792	**	0.0056
Clari/Ova vs. Hi/Ova	-1.405	*	0.0331
Clari/Ova vs. Hi/Clar/Ova	-3.378	****	< 0.0001
Clari/Ova vs. Hi/Clar/Ova/Dex	1.171	ns	0.094
Clari/Ova/Dex vs. Hi/Sal	2.288	**	0.0015
Clari/Ova/Dex vs. Hi/Ova	-0.9088	ns	0.1889
Clari/Ova/Dex vs. Hi/Clar/Ova	-2.882	***	0.0002
Clari/Ova/Dex vs. Hi/Clar/Ova/Dex	1.667	*	0.0289
Hi/Sal vs. Hi/Ova	-3.197	***	0.0001
Hi/Sal vs. Hi/Clar/Ova	-5.169	****	< 0.0001
Hi/Sal vs. Hi/Clar/Ova/Dex	-0.6204	ns	0.3441
Hi/Ova vs. Hi/Clar/Ova	-1.973	**	0.004
Hi/Ova vs. Hi/Clar/Ova/Dex	2.576	***	0.0008
Hi/Clar/Ova vs. Hi/Clar/Ova/Dex	4.549	****	< 0.0001

D

One way ANOVA (Macrophages)	Mean Diff.	Summary	P Value
Sal vs. Ova	-28.44	*	0.0219
Sal vs. Clari/Ova	-14.75	ns	0.2194
Sal vs. Clari/Ova/Dex	-4.77	ns	0.7048
Sal vs. Hi/Sal	-15.21	ns	0.2058
Sal vs. Hi/Ova	-29.28	*	0.0257
Sal vs. Hi/Clari/Ova	-32.21	*	0.0103
Sal vs. Hi/Clari/Ova/Dex	-16.17	ns	0.1793
Ova vs. Clari/Ova	13.69	ns	0.2535
Ova vs. Clari/Ova/Dex	23.67	ns	0.0674
Ova vs. Hi/Sal	13.23	ns	0.2693
Ova vs. Hi/Ova	-0.8414	ns	0.9467
Ova vs. Hi/Clari/Ova	-3.769	ns	0.7508
Ova vs. Hi/Clari/Ova/Dex	12.27	ns	0.3049
Clari/Ova vs. Clari/Ova/Dex	9.98	ns	0.4299
Clari/Ova vs. Hi/Sal	-0.4569	ns	0.9693
Clari/Ova vs. Hi/Ova	-14.53	ns	0.2531
Clari/Ova vs. Hi/Clari/Ova	-17.46	ns	0.148
Clari/Ova vs. Hi/Clari/Ova/Dex	-1.418	ns	0.9048
Clari/Ova/Dex vs. Hi/Sal	-10.44	ns	0.4093
Clari/Ova/Dex vs. Hi/Ova	-24.51	ns	0.0721
Clari/Ova/Dex vs. Hi/Clari/Ova	-27.44	*	0.0356
Clari/Ova/Dex vs. Hi/Clari/Ova/Dex	-11.4	ns	0.3681
Hi/Sal vs. Hi/Ova	-14.08	ns	0.268
Hi/Sal vs. Hi/Clari/Ova	-17	ns	0.1585
Hi/Sal vs. Hi/Clari/Ova/Dex	-0.9607	ns	0.9354
Hi/Ova vs. Hi/Clari/Ova	-2.928	ns	0.816
Hi/Ova vs. Hi/Clari/Ova/Dex	13.11	ns	0.3014
Hi/Clari/Ova vs. Hi/Clari/Ova/Dex	16.04	ns	0.1826

E



F

One Way ANOVA (5mg/ml Mch)	Mean Diff.	Summary	P Value
Saline vs. Ova	-105	***	0.0001
Saline vs. Clari/Ova	-16.92	ns	0.188
Saline vs. Clari/Ova/Dex	-50.6	***	0.0005
Saline vs. HiSal	7.261	ns	0.5082
Saline vs. Hi/Ova	-72.17	****	< 0.0001
Saline vs. Hi/Clari/Ova	-9.354	ns	0.4227
Saline vs. Hi/Clari/Ova/Dex	-33.34	**	0.0051
Ova vs. Clari/Ova	88.08	****	< 0.0001
Ova vs. Clari/Ova/Dex	54.4	***	0.0003
Ova vs. HiSal	112.3	****	< 0.0001
Ova vs. Hi/Ova	32.83	*	0.019
Ova vs. Hi/Clari/Ova	95.65	****	< 0.0001
Ova vs. Hi/Clari/Ova/Dex	71.66	****	< 0.0001
Clari/Ova vs. Clari/Ova/Dex	-33.68	*	0.0238
Clari/Ova vs. HiSal	24.18	ns	0.0646
Clari/Ova vs. Hi/Ova	-55.25	***	0.0006
Clari/Ova vs. Hi/Clari/Ova	7.561	ns	0.5679
Clari/Ova vs. Hi/Clari/Ova/Dex	-16.42	ns	0.2008
Clari/Ova/Dex vs. HiSal	57.86	***	0.0001
Clari/Ova/Dex vs. Hi/Ova	-21.57	ns	0.1353
Clari/Ova/Dex vs. Hi/Clari/Ova	41.24	**	0.0042
Clari/Ova/Dex vs. Hi/Clari/Ova/Dex	17.26	ns	0.1795
HiSal vs. Hi/Ova	-79.43	***	0.0004
HiSal vs. Hi/Clari/Ova	-16.62	ns	0.1603
HiSal vs. Hi/Clari/Ova/Dex	-40.6	***	0.001
Hi/Ova vs. Hi/Clari/Ova	62.81	***	0.0003
Hi/Ova vs. Hi/Clari/Ova/Dex	38.83	*	0.048
Hi/Clari/Ova vs. Hi/Clari/Ova/Dex	-23.98	*	0.0472

Supplementary Figure 7: Clarithromycin suppresses *Hi*-induced SSIAAD

A

One Way ANOVA (IL-5)	Mean Diff.	Summary	P Value
Sal vs. Ova	-4.370	***	0.0003
Sal vs. Clari/Ova	-3.605	***	0.0002
Sal vs. Clari/Ova/Dex	-4.693	****	< 0.0001
Sal vs. Hi/Sal	1.156	ns	0.2384
Sal vs. Hi/Ova	0.02027	ns	0.9833
Sal vs. Hi/Clari/Ova	-2.631	*	0.0103
Sal vs. Hi/Clari/Ova/Dex	-0.5396	ns	0.5387
Ova vs. Clari/Ova	0.765	ns	0.3849
Ova vs. Clari/Ova/Dex	-0.3229	ns	0.7392
Ova vs. Hi/Sal	5.526	****	< 0.0001
Ova vs. Hi/Ova	4.39	***	0.0001
Ova vs. Hi/Clari/Ova	1.739	ns	0.0804
Ova vs. Hi/Clari/Ova/Dex	3.83	***	0.0001
Clari/Ova vs. Clari/Ova/Dex	-1.088	ns	0.2487
Clari/Ova vs. Hi/Sal	4.761	****	< 0.0001
Clari/Ova vs. Hi/Ova	3.625	***	0.0005
Clari/Ova vs. Hi/Clari/Ova	0.9741	ns	0.3006
Clari/Ova vs. Hi/Clari/Ova/Dex	3.065	***	0.0009
Clari/Ova/Dex vs. Hi/Sal	5.849	****	< 0.0001
Clari/Ova/Dex vs. Hi/Ova	4.713	****	< 0.0001
Clari/Ova/Dex vs. Hi/Clari/Ova	2.062	ns	0.0507
Clari/Ova/Dex vs. Hi/Clari/Ova/Dex	4.153	****	< 0.0001
Hi/Sal vs. Hi/Ova	1.136	*	0.0170
Hi/Sal vs. Hi/Clari/Ova	-3.787	***	0.0008
Hi/Sal vs. Hi/Clari/Ova/Dex	-1.696	ns	0.0767
Hi/Ova vs. Hi/Clari/Ova	-2.651	*	0.0138
Hi/Ova vs. Hi/Clari/Ova/Dex	-0.5598	ns	0.5495
Hi/Clari/Ova vs. Hi/Clari/Ova/Dex	2.091	*	0.0312

B

One Way ANOVA (IL-13)	Mean Diff.	Summary	P Value
Sal vs. Ova	-2.112	***	0.0002
Sal vs. Clari/Ova	-0.6621	ns	0.1674
Sal vs. Clari/Ova/Dex	-1.104	*	0.0327
Sal vs. Hi/Sal	0.2404	ns	0.6254
Sal vs. Hi/Ova	-0.8983	ns	0.0773
Sal vs. Hi/Clari/Ova	-2.749	****	< 0.0001
Sal vs. Hi/Clari/Ova/Dex	-1.353	*	0.0158
Ova vs. Clari/Ova	1.45	**	0.0025
Ova vs. Clari/Ova/Dex	1.008	*	0.0349
Ova vs. Hi/Sal	2.352	****	< 0.0001
Ova vs. Hi/Ova	1.214	*	0.0128
Ova vs. Hi/Clari/Ova	-0.6367	ns	0.1702
Ova vs. Hi/Clari/Ova/Dex	0.7586	ns	0.132
Clari/Ova vs. Clari/Ova/Dex	-0.4421	ns	0.3109
Clari/Ova vs. Hi/Sal	0.9024	*	0.0454
Clari/Ova vs. Hi/Ova	-0.2363	ns	0.585
Clari/Ova vs. Hi/Clari/Ova	-2.087	****	< 0.0001
Clari/Ova vs. Hi/Clari/Ova/Dex	-0.6914	ns	0.1502
Clari/Ova/Dex vs. Hi/Sal	1.345	**	0.0065
Clari/Ova/Dex vs. Hi/Ova	0.2058	ns	0.6515
Clari/Ova/Dex vs. Hi/Clari/Ova	-1.645	**	0.0013
Clari/Ova/Dex vs. Hi/Clari/Ova/Dex	-0.2493	ns	0.6126
Hi/Sal vs. Hi/Ova	-1.139	*	0.0186
Hi/Sal vs. Hi/Clari/Ova	-2.989	****	< 0.0001
Hi/Sal vs. Hi/Clari/Ova/Dex	-1.594	**	0.0033
Hi/Ova vs. Hi/Clari/Ova	-1.85	**	0.004
Hi/Ova vs. Hi/Clari/Ova/Dex	-0.4551	ns	0.3585
Hi/Clari/Ova vs. Hi/Clari/Ova/Dex	1.395	**	0.0086

C

One Way ANOVA (IL-17)	Mean Diff.	Summary	P Value
Sal vs. Ova	-0.03272	ns	0.9556
Sal vs. Clari/Ova	0.04776	ns	0.9318
Sal vs. Clari/Ova/Dex	0.1406	ns	0.8112
Sal vs. Hi/Sal	-1.096	ns	0.0702
Sal vs. Hi/Ova	-4.45	****	< 0.0001
Sal vs. Hi/Clari/Ova	-1.074	ns	0.053
Sal vs. Hi/Clari/Ova/Dex	-1.669	**	0.0039
Ova vs. Clari/Ova	0.08049	ns	0.8853
Ova vs. Clari/Ova/Dex	0.1733	ns	0.7684
Ova vs. Hi/Sal	-1.064	ns	0.0785
Ova vs. Hi/Ova	-4.417	***	0.0001
Ova vs. Hi/Clari/Ova	-1.041	ns	0.0602
Ova vs. Hi/Clari/Ova/Dex	-1.636	**	0.0046
Clari/Ova vs. Clari/Ova/Dex	0.09284	ns	0.8679
Clari/Ova vs. Hi/Sal	-1.144	*	0.0477
Clari/Ova vs. Hi/Ova	-4.498	****	< 0.0001
Clari/Ova vs. Hi/Clari/Ova	-1.122	*	0.0325
Clari/Ova vs. Hi/Clari/Ova/Dex	-1.717	**	0.0018
Clari/Ova/Dex vs. Hi/Sal	-1.237	*	0.0426
Clari/Ova/Dex vs. Hi/Ova	-4.591	****	< 0.0001
Clari/Ova/Dex vs. Hi/Clari/Ova	-1.215	*	0.0301
Clari/Ova/Dex vs. Hi/Clari/Ova/Dex	-1.809	**	0.002
Hi/Sal vs. Hi/Ova	-3.354	****	< 0.0001
Hi/Sal vs. Hi/Clari/Ova	0.0225	ns	0.9666
Hi/Sal vs. Hi/Clari/Ova/Dex	-0.5723	ns	0.2913
Hi/Ova vs. Hi/Clari/Ova	3.376	***	0.0003
Hi/Ova vs. Hi/Clari/Ova/Dex	2.781	***	0.0004
Hi/Clari/Ova vs. Hi/Clari/Ova/Dex	-0.5948	ns	0.2217

Supplementary Figure 8: Clarithromycin works to suppress IL-17 responses in *Hi*-induced SSIAAD