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Streptococcus pneumoniae infection suppresses allergic airways disease by inducing regulatory T-cells

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ABSTRACT: An inverse association exists between some bacterial infections and the prevalence of asthma. We investigated whether *Streptococcus pneumoniae* infection protects against asthma using mouse models of ovalbumin (OVA)-induced allergic airway disease (AAD).

Mice were intratracheally infected or treated with killed *S. pneumoniae* before, during or after OVA sensitisation and subsequent challenge. The effects of *S. pneumoniae* on AAD were assessed.

Infection or treatment with killed *S. pneumoniae* suppressed hallmark features of AAD, including antigen-specific T-helper cell (Th) type 2 cytokine and antibody responses, peripheral and pulmonary eosinophil accumulation, goblet cell hyperplasia, and airway hyperresponsiveness. The effect of infection on the development of specific features of AAD depended on the timing of infection relative to allergic sensitisation and challenge. Infection induced significant increases in regulatory T-cell (Treg) numbers in lymph nodes, which correlated with the degree of suppression of AAD. Tregs reduced T-cell proliferation and Th2 cytokine release. The suppressive effects of infection were reversed by anti-CD25 treatment.

Respiratory infection or treatment with *S. pneumoniae* attenuates allergic immune responses and suppresses AAD. These effects may be mediated by *S. pneumoniae*-induced Tregs. This identifies the potential for the development of therapeutic agents for asthma from *S. pneumoniae*.

KEYWORDS: Asthma, allergic airways disease, immune modulation, regulatory T-cell, *Streptococcus pneumoniae*, suppression

Asthma is a major chronic respiratory illness that has dramatically increased in prevalence over the past 30 yrs. Prevalence rates have plateaued recently but remain high [1, 2]. Asthma is an inflammatory disease that is characterised by the infiltration of T-helper cell (Th) type 2 and eosinophils into the airway wall, and is associated with increased mucus production and airway hyperresponsiveness (AHR). The release of the Th2 cytokines interleukin (IL)-4, IL-5 and IL-13 by activated CD4+ Th2 cells is instrumental in disease pathogenesis, and mediates features of pathophysiology, including immunoglobulin (Ig)E production, eosinophil accumulation in blood and lungs, mucus hypersecretion and AHR [3]. In addition, Th2 cells, eosinophils and activated inflammatory cells release mediators that damage the mucosal epithelial lining and/or promote an exaggerated repair response, resulting in airway remodelling that contributes to irreversible airflow obstruction and chronic asthma.

Increases in asthma prevalence have been associated with reduced exposure to infectious agents. Recently, extensive research has focused on the significance of both the nature and timing of respiratory infections in asthma, and whether infection-induced skewing of the T-cell phenotype can influence the progression of asthma [4–8].

Although certain types of infections drive and/or exacerbate asthma [6–10], several studies support a role for some respiratory infections, such as mycobacterial infections, in suppressing Th2-driven allergic disease [4, 11]. Administration of mycobacterial components inhibits allergic airway

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disease (AAD); however, the efficacy of mycobacterial treatment in preventing allergic diseases in human trials has been varied, with decreases in the severity of atopic dermatitis but no affect on allergic asthma [11–13].

Streptococcus pneumoniae is commonly carried asymptomatically in the respiratory tract and is the predominant cause of community-acquired pneumonia in children and adults [14]. However, *S. pneumoniae* infection is not widely implicated in the pathogenesis of asthma. *S. pneumoniae* vaccination has been recommended to prevent this invasive *S. pneumoniae* disease in asthmatics, and asthma has been suggested as an independent risk factor for invasive *S. pneumoniae* disease. Nevertheless, there is evidence that *S. pneumoniae* infection or treatment may actually be beneficial in asthma [11, 14]. *S. pneumoniae* infection suppresses parasite-induced eosinophilia [15], suggesting that *S. pneumoniae* infection may have the potential to attenuate eosinophilic infiltration in asthma. Furthermore, *S. pneumoniae* vaccination of asthmatic children and elderly patients reduced the number and severity of asthmatic exacerbations [14].

In this study, we investigated the effect of *S. pneumoniae* infection on ovalbumin (OVA)-induced AAD in mice and examined the potential for the attenuation of asthma with killed *S. pneumoniae*. The role of regulatory T-cells (Tregs) in *S. pneumoniae*-mediated suppression of AAD was also investigated.

METHODS

Allergic sensitisation and challenge

To induce AAD, specific pathogen-free BALB/c mice were sensitised by *i.p.* injection of OVA (day 0, 50 μg; Sigma, St Louis, MO, USA) in Rehydrogel (1 mg; Reheis, Berkeley Heights, NJ, USA) and sterile saline (200 μL), and subsequently challenged (progression of AAD) by intranasal delivery of OVA (day 12–15, 10 μg in 50 μL sterile saline; figs 1–3) [8, 16, 17]. Mice were sacrificed and AAD was assessed 1 day later (day 16). Control (saline) groups received saline sensitisation and OVA challenge. All procedures were approved by the University of Newcastle animal ethics committee (Newcastle, Australia).

S. pneumoniae *inoculation*

Animals were infected or treated with *S. pneumoniae* intratracheally (IT) (figs 1–3) [16]. For treatment, mice were inoculated with ethanol-killed *S. pneumoniae* (three times, every 12 h). Killed *S. pneumoniae* was washed three times with saline to remove residual ethanol [18]. This protocol ensured that *S. pneumoniae* antigens were present in the lungs for the equivalent period as live *S. pneumoniae* infection. Control groups were sham-inoculated with saline. This did not alter any features of AAD and results were the same as for saline-sensitised groups; therefore, only results from saline-sensitised controls are presented.

T-cell cytokines, serum antibodies and inflammation

OVA-induced cytokine release by lung-draining mediastinal lymph node (MLN) T-cells, serum antibody titres, differential leukocyte counts in blood and bronchoalveolar lavage fluid (BALF), eosinophils in inflamed peribronchial tissue, and goblet cell hyperplasia were assessed as previously described [8, 16, 17].

Lung function

AHR was assessed by invasive plethysmography, as a change in airway function (transpulmonary resistance and dynamic compliance) following challenge with increasing doses of aerosolised methacholine [8, 16, 17].

Enumeration of Tregs

The numbers of CD4+ CD25+ FoxP3+ Tregs in MLNs were assessed by flow cytometry using the antibody conjugates CD4–fluorescein isothiocyanate, CD25–PerCP-Cy5.5 (both BD Biosciences, North Ryde, Australia) and FoxP3–phycoerythrin (eBioscience, San Diego, CA, USA) [19]. Cells stained with isotype-matched antibodies were used as controls.

Treg suppression assays

Treg function was assessed using suppression assays by co-culture of CD11c+ cells (2.5×10^4 cells), CD4+ CD25- effector T-cells (5×10^4 cells) and CD4+ CD25+ cells ($0–12 \times 10^3$ cells) purified from MLNs using an AutoMACs Pro (Miltenyi Biotec, Auburn, CA, USA) with OVA (5 μg·mL^{-1}) or anti-CD3 (1 μg·mL^{-1}; Biolegend, San Diego, CA, USA) [20]. The mean±SEM purity of sorted cells was determined by FAC to be 95±0.83% for CD11c+ cells, 97±0.87% for CD4+ CD25- effector cells and mean >81–6, SEM 0.45–0.90% for CD4+ CD25+ cells. Cytokine levels in supernatants were assessed [8, 16, 17].

Anti-CD25 treatment

Treg function was suppressed by treatment with anti-CD25 (100 μg in 200 μL saline, *i.p.*; PC61, eBioscience) 3 days before concurrent *S. pneumoniae* infection and OVA sensitisation [21].

Statistical analysis

Results are representative of two to three independent experiments and are presented as mean±SEM, where $n \geqslant 8$ individual mice. Suppression assays were performed in triplicate with pooled samples. AHR data were analysed by repeated-measures one-way ANOVA. p-values for AHR were determined for the entire dose–response curve. Other data were analysed by one-way ANOVA with Tukey's post-test.

RESULTS

Some results (supplementary figures 1–3) are presented as online supplementary material.

Induction of AAD

The development of AAD (OVA groups) resulted in significantly increased OVA-induced Th2 cytokine and IL-10 production by MLN and splenic T-cells, OVA-specific IgG1 and total IgE levels in serum, and recruitment of eosinophils into the blood, peribronchial spaces (BALF) and lung tissue, compared to nonallergic (saline) controls (figs 1–4) [8, 16, 17]. Allergic inflammation was associated with enhanced goblet cell hyperplasia in the airway epithelium and AHR (significantly increased transpulmonary resistance and decreased dynamic compliance) (figs 1–3) [8, 16, 17]. The same OVA control data are presented in figures 1 and 3.

S. pneumoniae *infection suppresses the progression of AAD*

We previously showed that maximal immune responses to *S. pneumoniae* infection occur 2 days after inoculation [22].

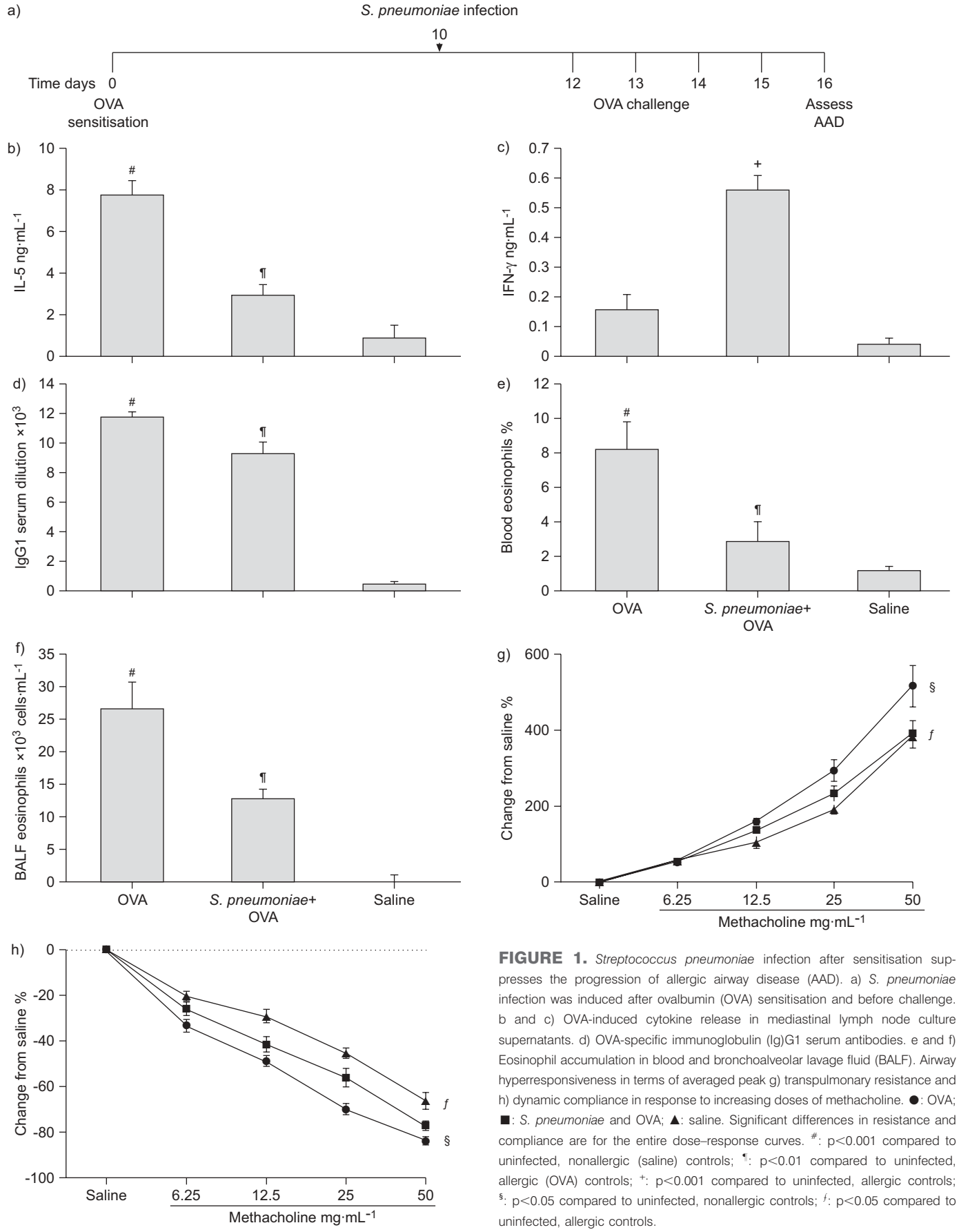

FIGURE 1. *Streptococcus pneumoniae* infection after sensitisation suppresses the progression of allergic airway disease (AAD). a) *S. pneumoniae* infection was induced after ovalbumin (OVA) sensitisation and before challenge. b and c) OVA-induced cytokine release in mediastinal lymph node culture supernatants. d) OVA-specific immunoglobulin (Ig)G1 serum antibodies. e and f) Eosinophil accumulation in blood and bronchoalveolar lavage fluid (BALF). Airway hyperresponsiveness in terms of averaged peak g) transpulmonary resistance and h) dynamic compliance in response to increasing doses of methacholine. ●: OVA; ■: *S. pneumoniae* and OVA; ▲: saline. Significant differences in resistance and compliance are for the entire dose–response curves. #: $p<0.001$ compared to uninfected, nonallergic (saline) controls; ¶: $p<0.01$ compared to uninfected, allergic (OVA) controls; +: $p<0.001$ compared to uninfected, allergic controls; §: $p<0.05$ compared to uninfected, nonallergic controls; *f*: $p<0.05$ compared to uninfected, allergic controls.

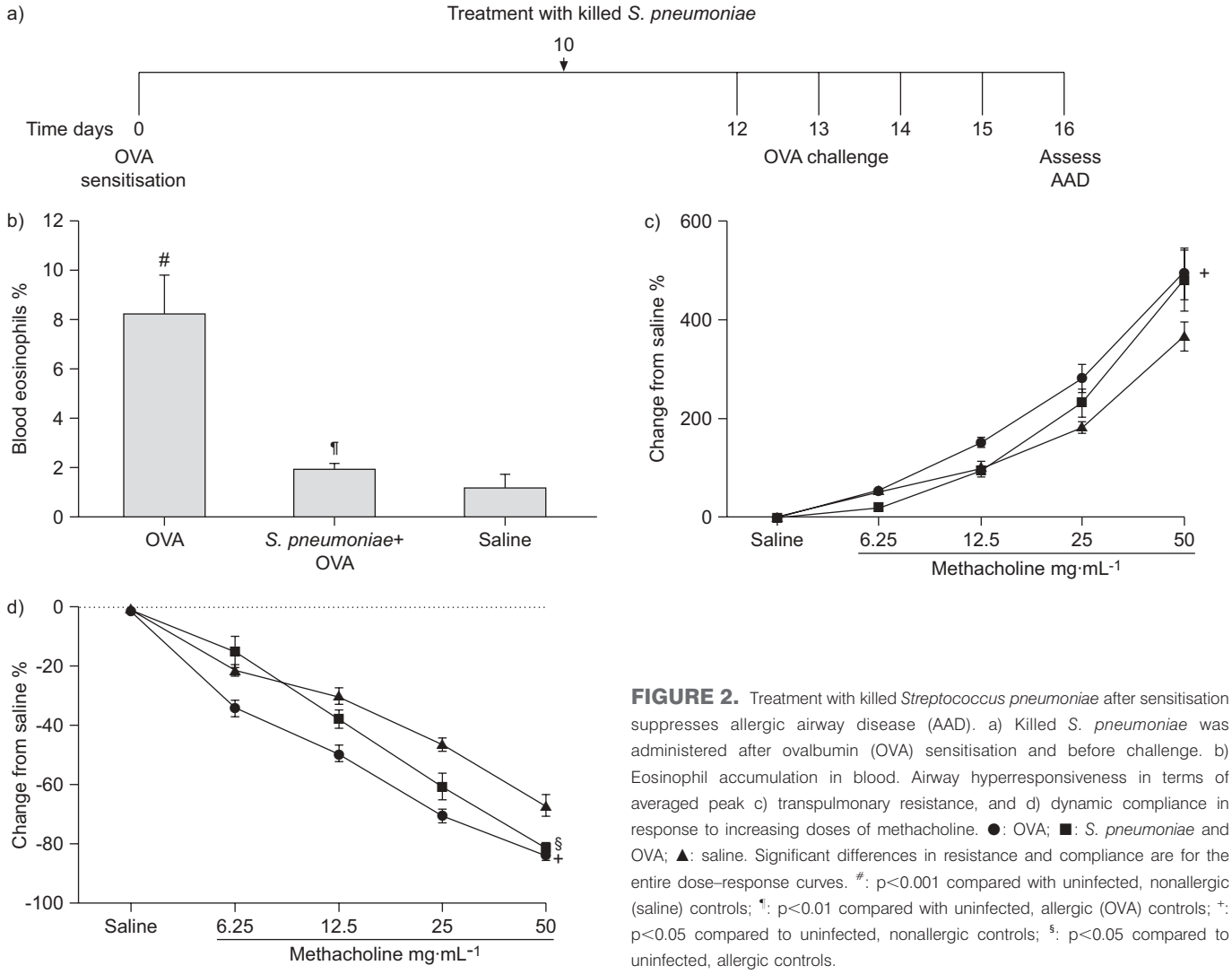

FIGURE 2. Treatment with killed *Streptococcus pneumoniae* after sensitisation suppresses allergic airway disease (AAD). a) Killed *S. pneumoniae* was administered after ovalbumin (OVA) sensitisation and before challenge. b) Eosinophil accumulation in blood. Airway hyperresponsiveness in terms of averaged peak c) transpulmonary resistance, and d) dynamic compliance in response to increasing doses of methacholine. ●: OVA; ■: *S. pneumoniae* and OVA; ▲: saline. Significant differences in resistance and compliance are for the entire dose–response curves. #: $p<0.001$ compared with uninfected, nonallergic (saline) controls; ¶: $p<0.01$ compared with uninfected, allergic (OVA) controls; +: $p<0.05$ compared to uninfected, nonallergic controls; §: $p<0.05$ compared to uninfected, allergic controls.

To investigate the effects of *S. pneumoniae* infection on the progression of AAD, OVA-sensitised mice were infected 2 days before challenge (fig. 1).

Infection (*S. pneumoniae* plus OVA groups) significantly suppressed OVA-induced IL-5 release from MLN T-cells, OVA-specific serum IgG1 titre, the numbers of eosinophils in the blood and BALF, and AHR (reduced resistance and increased compliance) compared to uninfected, allergic (OVA) controls. Notably, the levels of eosinophils in the blood were reduced to similar levels as those of the uninfected, nonallergic (saline) controls. By contrast, infection significantly increased OVA-induced interferon (IFN)-γ release from T-cells. OVA-specific IgG2a was not detected and infection had no effect on the numbers of neutrophils in BALF, eosinophils in peribronchial tissue or goblet cells in airway epithelium (data not shown).

Treatment with killed S. pneumoniae *suppresses the progression of AAD*

The protective properties of *S. pneumoniae* infection suggest that *S. pneumoniae* might have potential as a treatment for asthma. Therefore, we investigated whether a killed *S. pneumoniae* formulation could also inhibit the progression of AAD. OVA-sensitised mice were inoculated with whole ethanol-killed *S. pneumoniae* before OVA challenge and the progression of AAD was assessed (fig. 2).

Killed *S. pneumoniae* significantly decreased eosinophilic inflammation in the blood, as well as goblet cell hyperplasia, OVA-induced IL-5 and IFN-γ secretion by T-cells, and OVA-specific IgG1 production, compared to untreated, allergic controls [16]. AHR was also suppressed: there was a trend towards a reduction in resistance, although this was not statistically significant; and a suppression of compliance, which was statistically significant.

Resolved or concurrent S. pneumoniae *infection suppresses the induction of AAD*

Since *S. pneumoniae* infection and killed *S. pneumoniae* suppressed the progression of AAD in sensitised mice, we then investigated the effects of infection on sensitisation during the induction of AAD. Maximal immune responses occur 2 days after infection and recovery from inflammation is

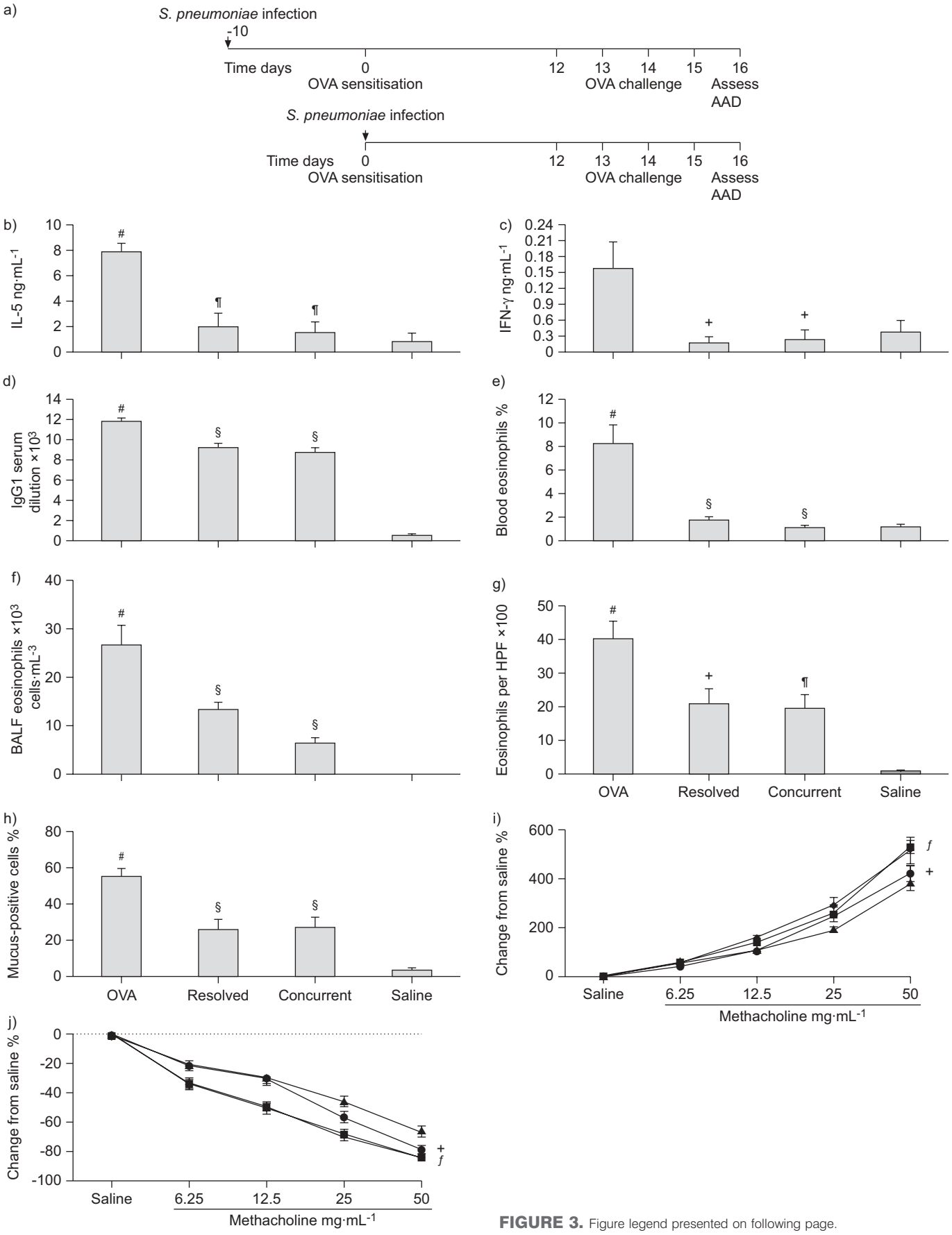

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FIGURE 3. Resolved or concurrent *Streptococcus pneumoniae* infection at sensitisation suppresses the induction of allergic airway disease (AAD). a) *S. pneumoniae* infection was induced before or during ovalbumin (OVA) sensitisation. b and c) OVA-induced cytokine release in mediastinal lymph node culture supernatants. d) OVA-specific immunoglobulin (Ig)G1 serum antibodies. e–g) Eosinophil accumulation in blood, bronchoalveolar lavage fluid (BALF) and tissue. h) Mucus-secreting cell numbers surrounding the airway lumen. Airway hyperresponsiveness in terms of averaged peak i) transpulmonary resistance and j) dynamic compliance in response to increasing doses of methacholine. ◆: OVA; ■: resolved; ●: concurrent; ▲: saline. Significant differences in resistance and compliance are for the entire dose–response curves. HPF: high-powered field. #: $p<0.001$ compared to uninfected, nonallergic (saline) controls; ¶: $p<0.01$ compared to uninfected, allergic (OVA) controls; +: $p<0.05$ compared to uninfected, allergic controls; §: $p<0.001$ compared to uninfected, allergic controls; ƒ: $p<0.05$ compared to uninfected, nonallergic controls.

complete after 10 days [16, 22]. Therefore, mice were infected either 10 days before (resolved infection) or during (concurrent infection) sensitisation to OVA (fig. 3). Infection concurrent with sensitisation ensured that maximal immunomodulatory effects of infection occurred during the sensitisation phase.

Resolved or concurrent infection significantly suppressed the levels of OVA-induced cytokine release from MLN T-cells, OVA-specific serum IgG1, eosinophils in blood, BALF and peribronchial tissue, and goblet cells in the airways, compared to uninfected, allergic controls. Resolved infection had no significant impact on AHR, whereas concurrent infection reduced AHR. IL-5, IFN-γ and blood eosinophils were suppressed to the same levels as in uninfected, nonallergic (saline) controls. Again, IgG2a was not detected and BALF neutrophil numbers did not vary between groups (data not shown).

Concurrent infection suppresses the induction of additional features of AAD

Since concurrent infection during sensitisation has the most potent suppressive effects on AAD, this model was used to investigate further the mechanisms that underpin suppression. First, the effects of concurrent infection on additional features of AAD were assessed (fig. 4).

Infection suppressed local OVA-induced IL-13 release from MLN T-cells, systemic OVA-induced IL-5, IL-13 and IFN-γ release from splenocytes, and total circulating IgE responses to the same level as uninfected, nonallergic controls. By contrast, infection increased both local and systemic IL-10 release. Transforming growth factor (TGF)-β was not detected in any sample.

Concurrent S. pneumoniae *infection increases the number of Treg cells in lymph nodes in AAD*

IL-10 is an immunosuppressive cytokine that may be released from T-cells or Tregs [23]. The increases in IL-10 in MLN and splenocyte cultures indicated that the suppressive effects of infection may result from the induction of Tregs. To investigate this possibility, mice were concurrently infected with *S. pneumoniae* during sensitisation and Tregs were enumerated in MLNs.

Infection increased both the numbers and percentages of CD4+ CD25+ FoxP3+ cells in MLNs compared to uninfected, allergic controls (fig. 5 and supplementary figure 1).

We then assessed whether infection after sensitisation (which had similar suppressive effects to concurrent infection) and resolved infection (which suppressed inflammation but not AHR) also altered numbers of Tregs in MLNs (supplementary figure 1). Infection after sensitisation also increased the numbers of Tregs in MLNs, which may be responsible for similar levels of suppression of AAD. Resolved infection did not significantly increase the numbers or percentages of CD4+ CD25+ FoxP3+ cells. It is likely that resolved infection induced Tregs at some time during the course of the induction and progression of AAD, which may account for the suppression of some features of AAD but not others.

S. pneumoniae *infection-induced Tregs suppress T-cell proliferation and Th2 cytokine release in AAD*

The function and suppressive mechanisms of infection-induced Tregs was assessed using T-cell suppression assays.

Tregs from the MLNs of mice with AAD with or without concurrent infection suppressed the proliferation of CD4+ CD25- T-cells from the same lymph nodes in the presence of either OVA or anti-CD3 (fig. 6). Tregs also suppressed the release of IL-5 and IL-13, and increased IL-10 production in response to OVA stimulation. However, infection did not enhance the suppressive function of Tregs on proliferation or cytokine release. This indicates that the suppression of AAD by infection occurs through increases in Treg numbers rather than by enhancing suppressive capacity.

Anti-CD25 treatment reverses the suppressive effects of infection on the induction of AAD

We then confirmed that *S. pneumoniae*-induced Treg cells were responsible for the suppressive effects of infection on AAD. Anti-CD25 was administered 3 days before concurrent infection and sensitisation, which completely inhibited the development of CD4+ CD25+ Foxp3+ Tregs (data not shown). The effects of administration on the suppression of AAD were assessed (fig. 7 and supplementary figure 2).

Anti-CD25 administration removed the infection-mediated suppression of OVA-induced IL-5, IL-13 and IFN-γ secretion by MLN and splenic T-cells, OVA-specific serum IgG1 production, the numbers of eosinophils in blood, BALF and peribronchial tissue, goblet cell hyperplasia, and AHR. Administration also reversed the increased OVA-induced release of IL-10 from MLN T-cells. Blocking Tregs in infected groups allowed the induction of AAD that was similar in severity to AAD in Treg-depleted, uninfected allergic groups. There were few differences between Treg-depleted and nondepleted allergic controls, indicating that effector T-cells were not affected by treatment. The administration of antibody isotype controls had no effect (supplementary figure 3). This demonstrates that it is the CD25+ Tregs, which are induced by infection, that mediate the suppression of AAD.

DISCUSSION

Using a mouse model of OVA-induced Th2-driven AAD, we demonstrate that *S. pneumoniae* infection, or whole killed *S. pneumoniae*, suppress the hallmark features of AAD. This included inhibition of: antigen-specific Th2 cytokine and antibody responses; peripheral and pulmonary eosinophil accumulation; goblet cell hyperplasia; and AHR. The effect of infection

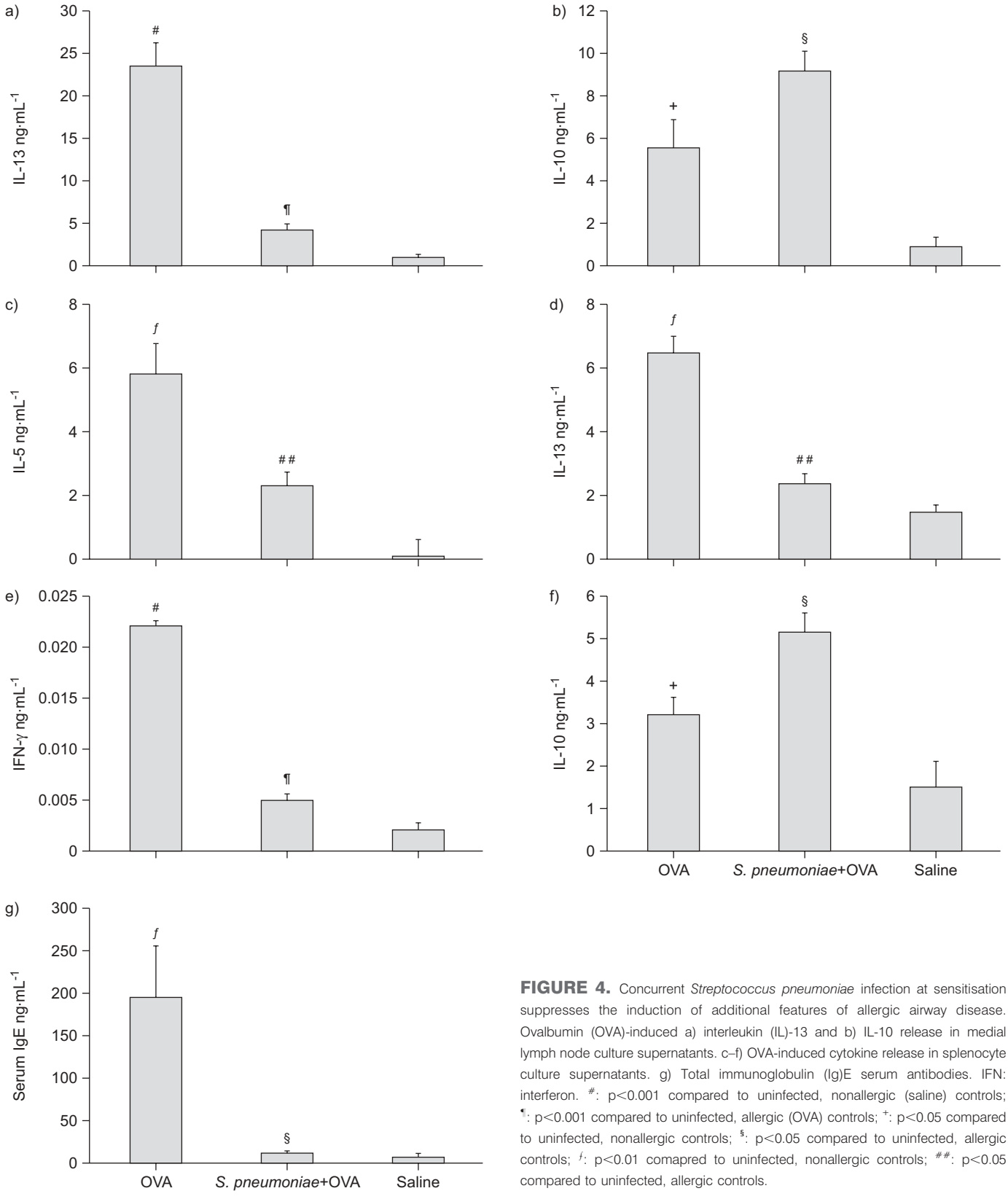

FIGURE 4. Concurrent *Streptococcus pneumoniae* infection at sensitisation suppresses the induction of additional features of allergic airway disease. Ovalbumin (OVA)-induced a) interleukin (IL)-13 and b) IL-10 release in medial lymph node culture supernatants. c–f) OVA-induced cytokine release in splenocyte culture supernatants. g) Total immunoglobulin (Ig)E serum antibodies. IFN: interferon. #: $p<0.001$ compared to uninfected, nonallergic (saline) controls; ¶: $p<0.001$ compared to uninfected, allergic (OVA) controls; +: $p<0.05$ compared to uninfected, nonallergic controls; §: $p<0.05$ compared to uninfected, allergic controls; *f*: $p<0.01$ comapred to uninfected, nonallergic controls; ##: $p<0.05$ compared to uninfected, allergic controls.

on some of the specific features of AAD was dependent on the timing of infection relative to allergic sensitisation and challenge. The attenuating effects of *S. pneumoniae* result from the immunosuppressive activity of increased numbers of Tregs that are induced by *S. pneumoniae*. These Tregs mediate suppression by reducing Th2 cell responses.

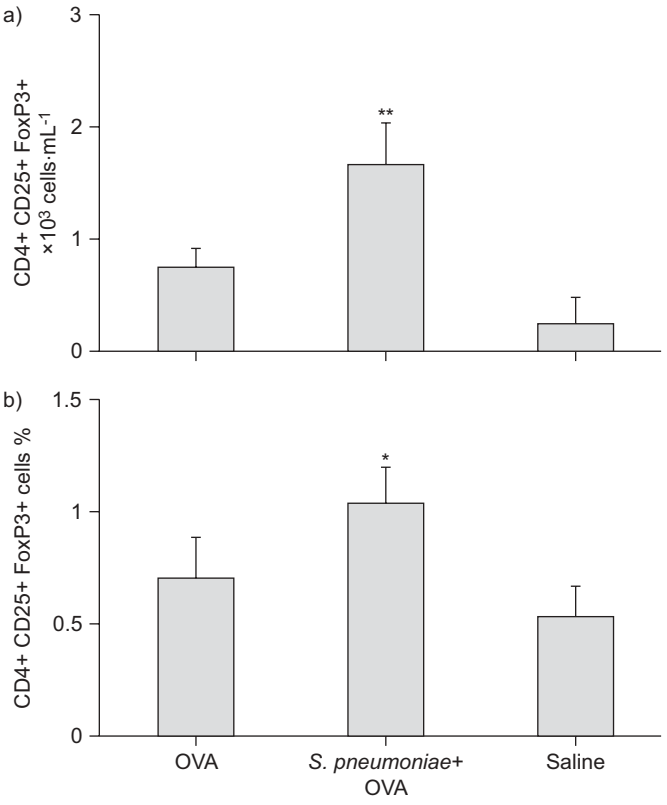

FIGURE 5. *Streptococcus pneumoniae* infection during sensitisation induces increases in regulatory T-cells (Tregs) in allergic airway disease. a) Total numbers and b) percentages of CD4+ CD25+ Foxp3+ Treg cells in medial lymph nodes. p-values represent significant differences compared to uninfected, allergic (ovalbumin; OVA) controls. *: $p<0.05$; **: $p<0.01$.

OVA-induced models of AAD have been widely employed to further the understanding of asthma pathogenesis (importance of Th2 cells and cytokines, involvement of IL-13 and IL-4R, role of eosinophils in remodelling), which has led to these molecules and events being targeted therapeutically in asthma [24]. In our model the IP delivery of OVA in alum drives the development of systemic Th2 responses and allergic sensitisation. Subsequent OVA challenge leads to the recruitment of Th2 cells into the airways that release cytokines, induce eosinophil influx and the progression of AAD. This model, therefore, enables the therapeutic manipulation of Th2-mediated allergic inflammation and the induction and progression of AAD.

We first examined whether infection would inhibit the progression of AAD in sensitised groups. Infection of sensitised mice before challenge significantly decreased the production of OVA-induced IL-5 from T-cells, eosinophil numbers in the blood and BALF, and AHR in AAD. This demonstrates that *S. pneumoniae* infection suppresses the progression of AAD and indicates the potential for the development of *S. pneumoniae* into a treatment for allergic asthma. However, infection significantly increased OVA-induced IFN-γ production and did not reduce the number of eosinophils in peribronchial tissue, or goblet cells in airway epithelium. *S. pneumoniae* infection elicits protective host immune responses that involve the release of IFN-γ. An active infection and subsequent IFN-γ production during OVA challenge may influence the expansion of T-cells, and lead to enhanced OVA-induced IFN-γ production. Generally, acute respiratory infections promote increased mucus secretion, which may be responsible for maintaining goblet cell hyperplasia in AAD in our studies.

S. pneumoniae infections result in serious health problems [14]; therefore, to investigate the potential for utilising *S. pneumoniae* as a therapeutic strategy for asthma, we examined the effect of treatment with killed *S. pneumoniae* on AAD. *S. pneumoniae* was ethanol-killed to maintain the Toll-like receptor-activating properties of *S. pneumoniae* components, which are lost during heat killing [18]. Treatment inhibited eosinophil influx into the blood and BALF, and goblet cell hyperplasia, and suppressed the development of AHR to similar levels to those of live infection. This suggests that it is the effects of *S. pneumoniae* antigens, rather than an inflammatory response to infection, that suppresses AAD.

The timing of infection relative to allergen sensitisation may be crucial in determining the effect on allergic outcomes [6, 7, 9]. Therefore, we then investigated the potential of resolved and concurrent *S. pneumoniae* infections to suppress the induction of AAD. A resolved infection significantly inhibited OVA-induced IL-5 and IFN-γ production from T-cells, eosinophil accumulation in blood, BALF and peribronchial tissue, and goblet cell hyperplasia, but had no significant effect on AHR, although a trend towards a reduction was observed. This indicates that the suppression of inflammation and AHR may occur independently, which has also been observed clinically. We speculate that the attenuation of Th2 immune responses by infection may be sustained, which accounts for the reduction in Th2 cytokines, eosinophils, and goblet cells, whilst the mechanisms of direct suppression of AHR are short-lived. Alternatively, there may be a temporal dissociation between modulatory (*e.g.* Treg) responses that are induced following infection, and the onset and persistence of AHR [25]. By contrast, concurrent infection with OVA sensitisation resulted in significant inhibition of all of the features of AAD assessed. In addition, concurrent infection suppressed OVA-induced IL-13 release from MLNs, IL-5, IL-13 and IFN-γ release from splenocytes, and total circulating IgE, but increased the release of IL-10.

Collectively, our results demonstrate that *S. pneumoniae* infection suppresses key features of AAD, irrespective of the timing of infection. Importantly, from a clinical perspective, infection at all three treatment times inhibited OVA-induced IL-5 release from T-cells and blood eosinophilia. This is particularly significant, as it reflects an attenuation of allergen-induced eosinophil expansion in the bone marrow. Eosinophils actively contribute to airway wall remodelling in allergic asthma in humans [26]. Suppression of blood eosinophilia by *S. pneumoniae* infection may be significant for the long-term inhibition of eosinophil accumulation in the lungs, leading to the inhibition or resolution of pathogenesis and remodelling events [27]. Thus, the features of AAD that were not reduced in the short term by infection may be resolved in the longer term. The suppressive effects of *S. pneumoniae* were observed with exposure before, during and after sensitisation. This indicates the potential application of *S. pneumoniae* to humans for both the

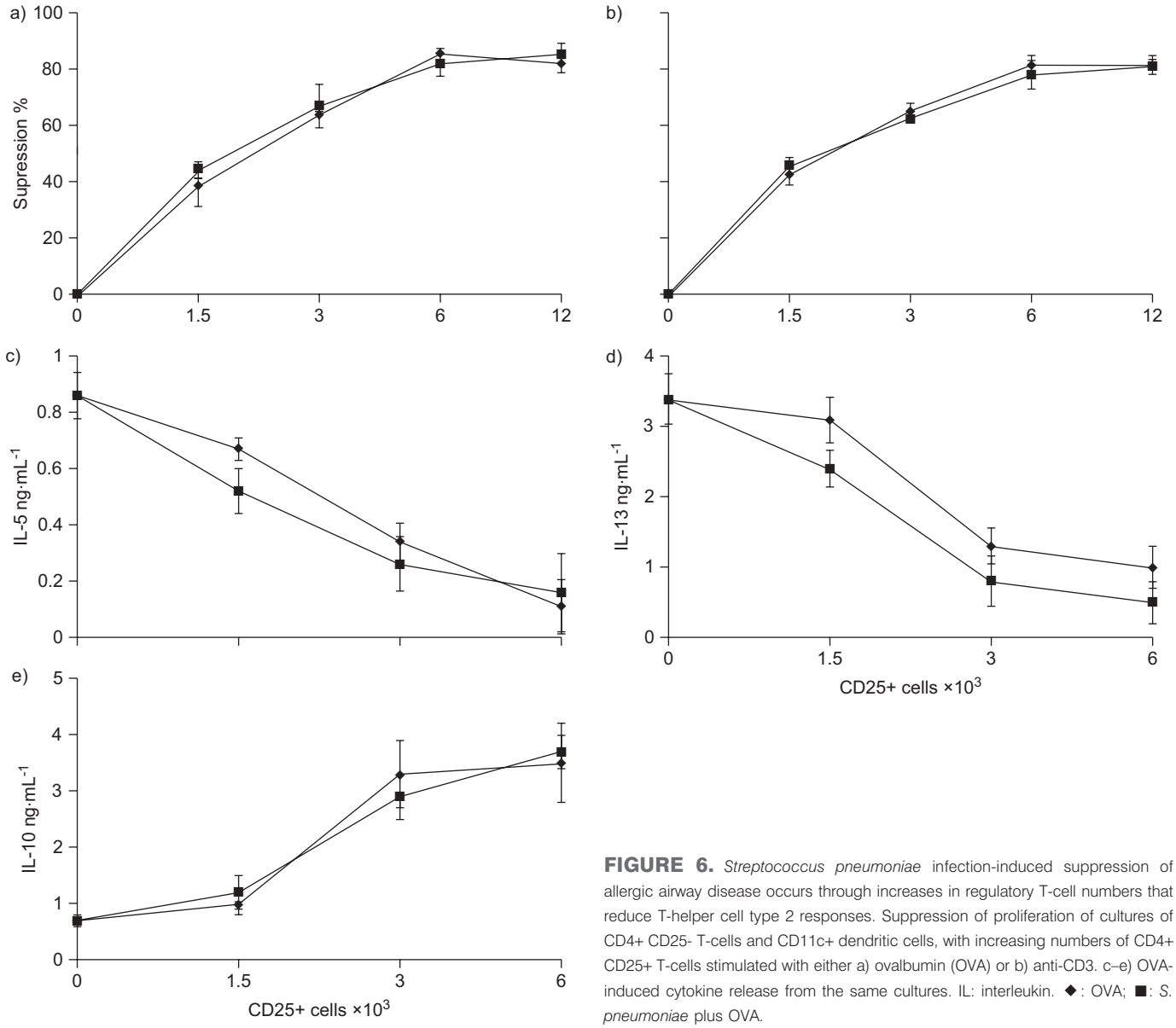

FIGURE 6. *Streptococcus pneumoniae* infection-induced suppression of allergic airway disease occurs through increases in regulatory T-cell numbers that reduce T-helper cell type 2 responses. Suppression of proliferation of cultures of CD4+ CD25- T-cells and CD11c+ dendritic cells, with increasing numbers of CD4+ CD25+ T-cells stimulated with either a) ovalbumin (OVA) or b) anti-CD3. c–e) OVA-induced cytokine release from the same cultures. IL: interleukin. ◆: OVA; ■: *S. pneumoniae* plus OVA.

suppression of sensitisation in naïve individuals and disease progression in sensitised patients. The beneficial effects of infection or treatment could be enhanced by the identification of the active components of *S. pneumoniae* that are responsible for the suppressive effects [11]. These components could be delivered repeatedly and in higher concentrations to enhance the suppression of the induction and progression of asthma. The identification of these components is the subject of ongoing studies.

Suppression of AAD by *S. pneumoniae* involves Tregs, which are significantly increased by concurrent infection. Increases in Tregs were reduced when infection had resolved prior to sensitisation and correlated with reduced suppression of AHR. Infection-induced Tregs suppressed the proliferation of both OVA-specific and anti-CD3 stimulated T-helper cells, and attenuated OVA-induced Th2 cytokine release.

Importantly, the suppression of all features of AAD by concurrent infection was removed by anti-CD25 treatment. Indeed, blocking Tregs in infected mice allowed the induction of AAD that was similar in severity to AAD induced in Treg-depleted groups without infection. CD25+ is also a marker of T-cell activation and anti-CD25 treatment may also deplete CD25+ effector T-cells. However, anti-CD25-treated, uninfected, allergic groups still developed AAD. This shows that effector T-cells were not depleted and that the effects in infected groups were the result of reduced Treg responses. The mechanisms through which Tregs suppressed proliferation and Th2 responses remain to be determined and are under further investigation. IL-10, but not TGF-β, levels were increased in MLN and splenocyte cultures and the possibility remains that suppression could be occurring through either the release of soluble factors or cell contact-dependent processes.

Our results show that *S. pneumoniae* may attenuate AAD by increasing Tregs in the local MLNs that suppress the proliferation of Th2 cells and eosinophils. Others have shown that the development and mobilisation of CD4+ CD25+ Tregs is induced

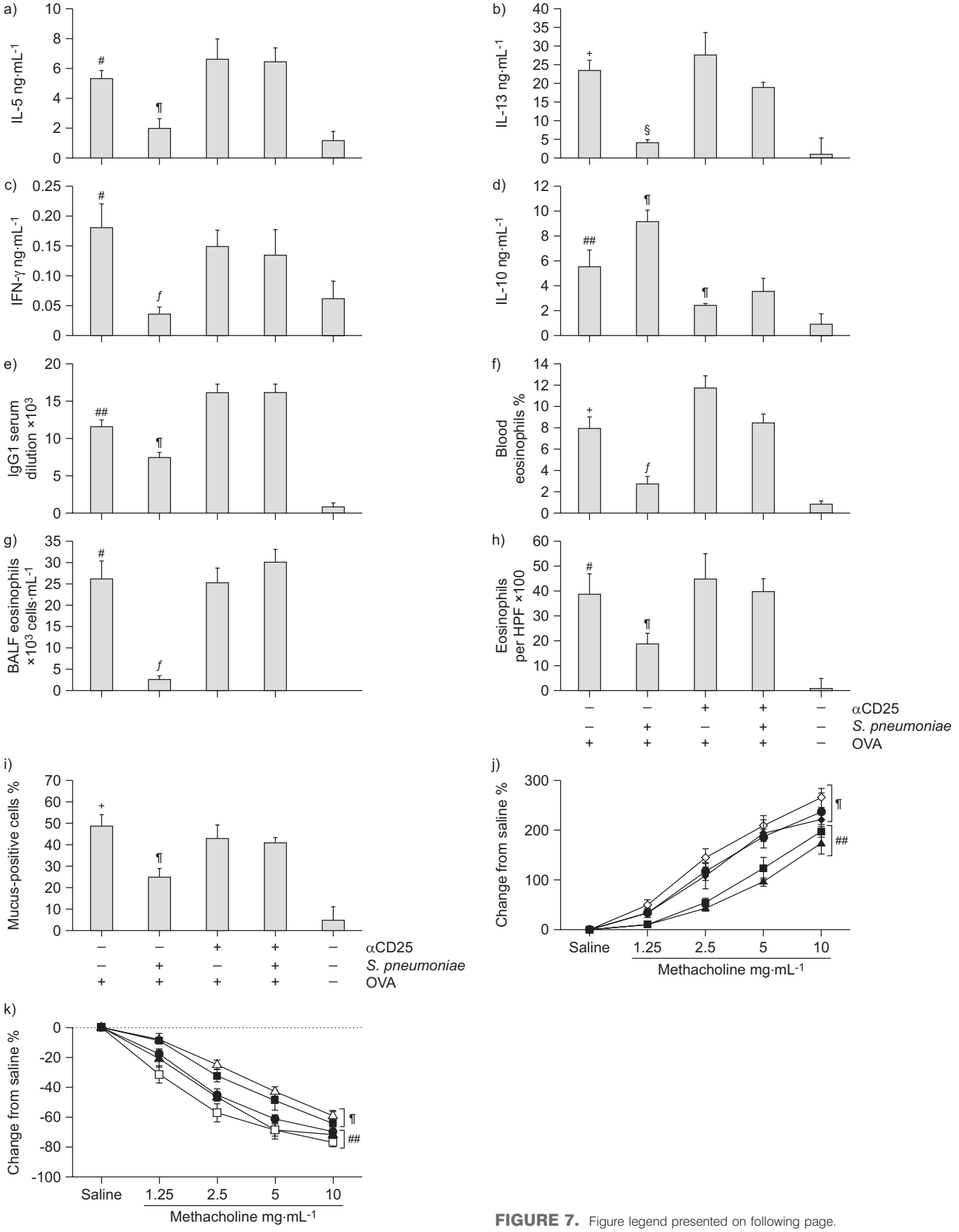

FIGURE 7. Figure legend presented on following page.

FIGURE 7. Anti-CD25 (αCD25) treatment reverses the suppressive effects of *Streptococcus pneumoniae* infection on allergic airway disease. a) ovalbumin (OVA)-induced cytokine release in medial lymph node culture supernatants. b) OVA-specific immunoglobulin (Ig)G1 serum antibodies. c) Eosinophil accumulation in blood, bronchoalveolar lavage fluid (BALF) and tissue. d) Mucus-secreting cell numbers surrounding the airway lumen. Airway hyperresponsiveness in terms of averaged peak e) transpulmonary resistance and f) dynamic compliance in response to increasing doses of methacholine. ◆: OVA; ■: *S. pneumoniae* plus OVA; ●: αCD25 plus *S. pneumoniae* plus OVA; □: αCD25 plus OVA; △: saline. Significant differences in resistance and compliance are for the entire dose–response curves. IL: interleukin; IFN: interferon; HPF: high-powered field. $^{\#}$: $p<0.01$ compared to uninfected, nonallergic (saline) controls; ¶: $p<0.05$ compared to uninfected, allergic (OVA) controls; $^{+}$: $p<0.001$ compared to uninfected, nonallergic controls; §: $p<0.001$ compared to uninfected, allergic controls; ^{f}: $p<0.01$ compared to uninfected, allergic controls; $^{\#\#}$: $p<0.05$ compared to uninfected, nonallergic controls.

by *S. pneumoniae* infection to a substantially greater extent than by other Gram-positive bacteria, and that Tregs are critical regulators of responses to *S. pneumoniae* infection [28]. Others have also shown that Tregs suppress the hallmark features of AAD [29]. Adoptive transfer of CD4+ CD25+ Tregs attenuated the activity of allergen-specific T-cells *in vivo*, leading to reduced Th2 cytokine expression in the lung, eosinophil recruitment and AHR. Thus, Tregs may inhibit overly exuberant inflammatory responses that occur during *S. pneumoniae* lung infection and may also be important in suppressing AADs and asthma.

Immunomodulatory therapy of asthma by increasing Th1 or inhibiting Th2 responses has received considerable attention. This work has drawn on widespread acceptance of the hygiene hypothesis, which is a well recognised, if oversimplified, link between infection and allergy. Administration of mycobacteria and other Th1-inducing infectious agents for the treatment of asthma in humans has given disappointing results, and successful results were dependent on the age of study subjects [4, 12, 14]. The therapeutic use of *S. pneumoniae* antigens that may prevent Th2 and eosinophil development and mobilisation by a different mechanism, *i.e.* the induction of Tregs, may be more effective in suppressing disease, and may potentially promote the resolution of tissue remodelling of the airways and persistent AHR in chronic asthma [11].

In summary, the significance of common respiratory bacterial infections in asthma is controversial. The present study is the first to directly examine the effect of respiratory *S. pneumoniae* infection on AAD. *S. pneumoniae* infection before challenge, or before or during sensitisation, with Th2-inducing antigen suppresses hallmark features of AAD. *S. pneumoniae* infection and treatment has the potential to inhibit allergic immune responses in asthmatics, including eosinophilia, that are linked to ongoing remodelling of the airway in asthma. This indicates the potential for the development of novel *S. pneumoniae*-based therapeutic agents for asthma. A greater understanding of the fundamental mechanisms associated with the suppression of AAD may lead to new approaches to the prevention and treatment of this disorder.

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STATEMENT OF INTEREST

None declared.

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