Neonatal Chlamydial Infection Induces Mixed T-Cell Responses That Drive Allergic Airway Disease

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Rationale: Chlamydial lung infection has been associated with asthma in children and adults. However, how chlamydial infection influences the development of immune responses that promote asthma remains unknown.

Objectives: To determine the effect of chlamydial infection at various ages on the development of allergic airway disease (AAD).

Methods: Mouse models of chlamydial lung infection and ovalbumin-induced AAD were established in neonatal and adult BALB/c mice. Neonatal or adult mice were given a chlamydial infection and 6 weeks later were sensitized and subsequently challenged with ovalbumin. Features of AAD and inflammation were compared between uninfected or unsensitized controls.

Measurements and Main Results: Mild *Chlamydia*-induced lung disease was observed 10–15 days after infection, as evidenced by increased bacterial numbers and histopathology in the lung and a reduction in weight gain. After 6 weeks, infection and histopathology had resolved and the rate of weight gain had recovered. Neonatal but not adult infection resulted in significant decreases in interleukin-5 production from helper T cells and by the numbers of eosinophils recruited to the lung in response to ovalbumin exposure. Remarkably, the effects of early-life infection were associated with the generation of both type 1 and 2 ovalbumin-specific helper T-cell cytokine and antibody responses. Furthermore, although neonatal infection significantly attenuated eosinophilia, the generation of the mixed T-cell response exacerbated other hallmark features of asthma: mucus hypersecretion and airway hyperresponsiveness. Moreover, infection prolonged the expression of AAD and these effects were restricted to early-life infection.

Conclusions: Early-life chlamydial infection induces a mixed type 1 and 2 T-cell response to antigen, which differentially affects the development of key features of AAD in the adult.

Keywords: asthma; infection; immunity; *Chlamydia*; T cells

Asthma has dramatically increased over the last 20 years in affluent societies (1). Although the causes of this increase remain unclear, aberrant T-cell responses to environmental

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Chlamydial infection is clinically associated with the onset and exacerbation of asthma in adults and children. However, it is unknown how this Th1-inducing infection is linked with Th2-driven asthma.

What This Study Adds to the Field

Chlamydia infection in early life can differentially drive key features of allergic airway disease. The effects of infection are associated with the generation of both Th1 and Th2 responses to an unrelated Th2-inducing antigen.

antigens play pivotal roles in disease development (2, 3). Indeed, allergen-specific CD4$^+$ helper T-cell type 2 (Th2) lymphocytes have been linked to the development of hallmark features of allergic asthma including airway eosinophil accumulation, mucus cell hyperplasia/metaplasia, and airway hyperresponsiveness (AHR) (3, 4).

The mechanisms underlying the development of aberrant Th2 responses in the airways are unknown but may involve a reduction in microbial exposure and lack of appropriate conditioning of immune responses to environmental antigens during maturation (5–7). The hygiene hypothesis (7) suggests that early-life infections are important in shaping dominant immune responses and that neonates must encounter Th1-inducing microbes to develop the ability to mount strong Th1 responses in later life (2). Reduced exposure may promote the persistence of the neonatal Th2 phenotype, which alters subsequent immune programming to environmental stimuli and predisposes to allergy and asthma. However, whether an infection in early life drives beneficial Th1 responses depends on the nature of the infection (microbial type, infectious load) and age at infection (reviewed by Hansbro and coworkers [8] and Openshaw and colleagues [9]). Studies in mice show that infection with respiratory syncytial virus in early life primes for Th2 responses to reinfection later in life (3), whereas mycobacterial infection in adults may cause a shift from Th2 to Th1 immune responses (10). Significantly, no experimental studies have investigated the effect of early-life Th1-inducing bacterial infections on the developing immune system and the subsequent impact on the induction of Th2 responses to inhaled antigens. It is known, however, that neonatal immunity is limited in its ability to generate strong Th1 responses to infection (11–13).

In humans and adult mice chlamydial infections promote strong Th1 responses, which effectively clear the bacteria. Only

in some predisposed adult individuals does infection induce Th2 responses (14–23). Chlamydial vaccination of neonatal mice results in Th1-mediated protection against reinfection in adulthood (24).

Strong evidence links *Chlamydia* (*Chlamydophila*) *pneumoniae* infection with the development and exacerbation of asthma in adults (reviewed by Hansbro and colleagues [8] and von Hertzen and coworkers [25]). Clinical studies also link infection by *C. pneumoniae* with wheezing and asthma in children (26–31).

C. pneumoniae has, therefore, been associated with both protective (Th1) as well as proasthmatic (Th2) immune responses. However, it remains unknown whether chlamydial infection in early life stimulates protective immunity or drives Th2 responses leading to the development and/or exacerbation of asthma later in life.

In this investigation we developed murine models of neonatal and adult chlamydial lung infection and of ovalbumin (Ova)-induced allergic airway disease (AAD) in BALB/c mice. We employed these models to determine the impact of early-life and adult chlamydial infection on the subsequent development of AAD in adulthood. Some of the results of these studies have been previously reported in the form of abstracts (32–36).

METHODS

See the online supplement for additional details on the methods used.

Experimental Models

Pregnant or 5-week-old virgin female BALB/c mice were obtained from the central animal house and used with approval from the animal ethics committee, University of Newcastle (Newcastle, Australia). Within 24 hours of birth or at 6 weeks (Day 0 of experimental models) mice were infected intranasally with *Chlamydia muridarum* (400 or 100 inclusion-forming units, ATCC VR-123, in 5 or 30 μl of sucrose–phosphate–glutamate buffer [SPG], respectively). Six weeks later (Day 45) mice were sensitized to Ova by intraperitoneal injection (50 μg of Ova [Sigma, Castle Hill, Australia] and 1 mg of Rehydragel [Reheis, Berkeley Heights, NJ] in 200 μl of 0.9% sterile saline [Sal]) (37) (Figure 1). Twelve days after sensitization mice were challenged intranasally with Ova (10 μg, 50 μl of phosphate-buffered saline, for consecutive days). One day later, mice were killed by sodium pentobarbital overdose (Abbott Australasia, Kurnell, Australia) and features of AAD were characterized. Controls received (*1*) SPG (intranasally), (*2*) *C. muridarum* infection (intranasally), (*3*) Ova (intraperitoneally), or (*4*) Sal (intraperitoneally) followed by intranasal Ova challenge.

Chlamydial Infection

Mice were weighed and rate of weight gain was calculated (g/d) throughout the experiments as a measure of health status. Chlamydial numbers in lungs were determined by real-time polymerase chain reaction (PCR) (38) and tissue culture (39).

Histopathology, Tissue Eosinophils, and Mucus-secreting Cells

Lungs were perfused, inflated, fixed, embedded, and sectioned (4–6 μm) (40). Sections were stained with hematoxylin and eosin (for histopathol-

ogy), chrome salt fixation (for eosinophils), or periodic acid–Schiff (for mucus-secreting cells). Histopathology was scored according to a set of custom-designed criteria (*see* the online supplement). Eosinophils and mucus-secreting cells were enumerated in inflamed airways as previously described (41).

T-cell Cytokines

Mediastinal lymph node cells were restimulated with Ova (200 μg) for 72 hours (38). Concentrations of IL-5, IL-13, and IFN-γ were determined in supernatants, using OptEIA mouse ELISA kits (BD Biosciences, North Ryde, Australia).

Serum Antibodies

Ova-specific IgG1:IgG2a ratios in serum were determined by ELISA (38), using Ova (2 μg/well) as the capture antigen.

Cytocentrifugation

Bronchoalveolar lavage fluid (BALF, 2 ml) was prepared and total cell numbers were determined with a hemocytometer (41). Cells prepared by cytocentrifugation (Shandon Cytospin; Thermo Fisher Scientific, Waltham, MA) were stained with May-Grünwald-Giemsa and leukocytes were enumerated on the basis of morphologic criteria (200 cells by light microscopy [×40]) (41).

Lung Function

Mice were anesthetized (ketamine and xylazine [80–100 and 10 mg/kg, respectively]; Troy Laboratories, Smithfield, Australia) and the tracheas were cannulated. Each cannula was connected to an inline aerosol administrator and ventilator, which were attached to a preamplifier and computer (Buxco, Wilmington, NC) to analyze pressure and flow waveforms and to determine airway resistance and dynamic compliance (42). Mice were nebulized with saline followed by increasing doses of methacholine (Sigma).

Statistics

Results are presented as means ± SEM from four to eight mice, in duplicate. The Wilcoxon rank-sum test was used for nonparametric tests (Mann-Whitney test for two independent samples). Comparison of airway resistance and dynamic compliance between groups was performed by one-way repeated measures analysis of variance. All analyses were performed with the intercooled Stata 8.2 statistical package (StataCorp, College Station, TX).

RESULTS

Chlamydial Lung Infection

The time course and profiles of chlamydial lung infection (growth and clearance) and subsequent histopathological damage and recovery associated with inflammation were similar in early life and adulthood (Figure 2). Chlamydial numbers in lungs were significantly increased 10 days (neonate and adult) and 15 days (neonate) after initial inoculation ($P < 0.05$ or $P < 0.01$) (Figure 2A). Infection was associated with a significant increase in histopathology score ($P < 0.01$, Days 10–15), which was characterized by the presence of foci of perivascular and peribronchiolar inflammatory infiltrates (predominantly neutrophils and mononuclear cells). Infection was resolved within 3 to 4 weeks, which
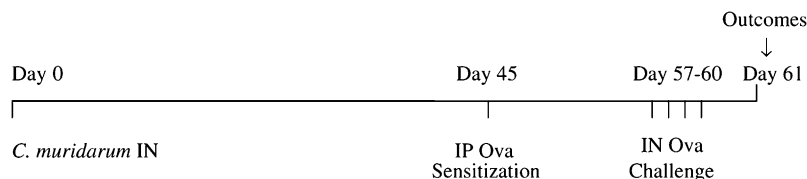
Outcomes
↓

Day 0   Day 45   Day 57-60   Day 61

C. muridarum IN   IP Ova Sensitization   IN Ova Challenge

Figure 1. Study protocols. Mice were infected with *Chlamydia muridarum* and allergic airway disease was induced by systemic (intraperitoneal [IP]) sensitization followed by intranasal (IN) challenge. Controls received (*1*) chlamydial vehicle (sucrose–phosphate–glutamate buffer [SPG]), (*2*) infection without ovalbumin (Ova) exposure, or sensitization with (*3*) Ova or (*4*) saline (vehicle for Ova) and Ova challenge without infection. Determinations of outcomes were made after the final Ova challenge.
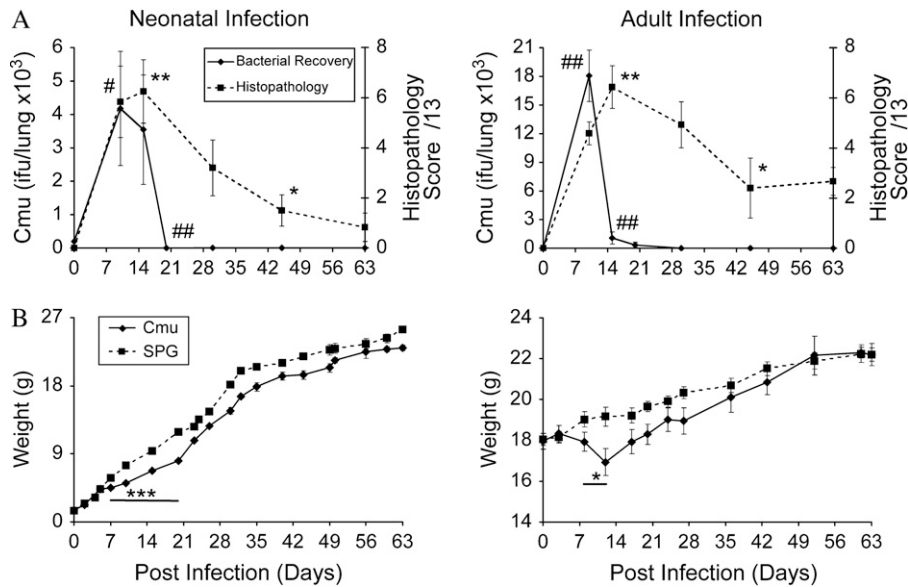
Figure 2. *C. muridarum* lung infection. Mice were infected in the first day of life (*left*) or at 6 weeks of age (*right*) and lung infection and histopathology (*A*) and weight (*B*) were determined over a 9-week time course. Significant differences in chlamydial (Cmu) numbers between Days 0 and 10 and between Days 15 and 20 are shown as #$P < 0.05$, ##$P < 0.01$. Significant differences in histopathology between Days 0 and 15 and between Days 15 and 42 are shown as *$P < 0.05$, **$P < 0.01$. Significant differences in the rate of weight gain between Days 7 and 23 (infected neonates) and between Days 8 and 12 (infected adults) of infected (Cmu) mice compared with uninfected (SPG) mice are shown as *$P < 0.05$, ***$P < 0.001$. ifu = inclusion-forming units; SPG = sucrose–phosphate–glutamate buffer.

was determined by both real-time PCR (Figure 2A) and tissue culture (data not shown). Histopathology was also largely resolved with only minimal cellular infiltrates apparent 6 and 9 weeks after infection (i.e., the time of sensitization and subsequent airway challenge with Ova). There was no increase in the numbers of mucus-secreting cells 10, 30, 42, or 61 days after infection. Significant decreases in the rate of weight gain were observed in neonates between Days 7 and 23 (0.28 ± 0.02 g/d) and in adults between Days 8 and 12 (-0.28 ± 0.07 g/d) compared with uninfected groups (0.40 ± 0.02 g/d, $P \leqslant 0.001$ and 0.04 ± 0.05 g/d, $P < 0.05$, respectively), which correlated with the peaks of infection (Figure 2B). The rate of weight gain between infected and uninfected groups was not significantly different during the later parts of the time course (Days 27–63 in neonates and Days 17–63 in adults). Thus the inflammatory and tissue lesions were largely resolved at the time of Ova exposure.

Effect of Infection on Lymph Node T-cell Cytokine and Serum Antibody Responses

The intraperitoneal model of AAD induced the hallmark features of Th2-driven AAD including robust type 2 cytokine responses from T cells, eosinophil influx into the lung, mucus hypersecretion in the airway wall, and development of AHR. Mediastinal lymph node T cells (from sensitized mice) stimulated with Ova released IL-5 (523 ± 106 pg/ml) and IL-13 (395 ± 70 pg/ml) but only background levels of IFN-γ (30 ± 8 pg/ml), when compared with cultures from saline controls (Sal) (Figures 3A–3C, Ova). A strong Th2 response was also characterized by the generation of high Ova-specific IgG1:IgG2a ratios (16 ± 3:1) in the Ova-sensitized and challenged group (Figure 3D, Ova). When mice were infected as neonates or adults, before intraperitoneal sensitization (Cmu/Ova), Ova-specific T-cell cytokine profiles were altered and IL-5 and IL-13 production levels were significantly reduced ($P < 0.05$ or $P < 0.01$; Figures 3A and 3B). Importantly, neonatal infection resulted in a dramatic increase in IFN-γ ($P < 0.01$) responses; by contrast, however, an adult infection had no effect on Ova-specific IFN-γ production (Figure 3C). This skew to an Ova-specific Th1 response after neonatal but not adult infection was further reflected by a significant decrease in Ova-specific IgG1:IgG2a ratios ($P \leqslant 0.001$, Cmu/Ova [neo]; Figure 3D). The ability of early-life infection to alter the way in which the T-cell response to Ova is generated after systemic exposure in the presence of adjuvant

(Rehydragel) highlights the capacity of this infection to alter the subsequent development of adaptive T-cell responses to unrelated antigens.

Effect of Infection on Granulocyte Recruitment, Mucus-secreting Cells, and Histopathology

Associated with the robust Th2 responses in the development of AAD were the following: eosinophil accumulation in airways and lung tissues ($18.9 \times 10^4 \pm 3.8 \times 10^4$ cells/ml of BALF and 20.1 ± 3.0 cells/100 µm adjacent to airways, respectively), an increase in mucus-secreting cell numbers surrounding airways (9.9 ± 3.1 cells/100 µm adjacent to airways), and marked increase in histopathology score (7.25 ± 0.45/13) compared with controls [Sal]) (Figures 4A–4E, Ova). By contrast to eosinophil numbers, neutrophil recruitment was limited ($2.1 \times 10^4 \pm 0.8 \times 10^4$ ml of BALF) in the Ova-sensitized group (Figure 4C, Ova). In groups exposed to neonatal chlamydial infection before sensitization (Cmu/Ova), the eosinophil (in BALF [$P < 0.001$] and tissue [$P < 0.05$]) and neutrophil (in BALF, $P < 0.001$) influx in response to Ova was markedly attenuated (Figures 4A–4C). By contrast, the number of mucus-secreting cells surrounding airways was significantly increased ($P < 0.01$) and the histopathology score was not affected, compared with Ova treatment alone (Figures 4D and 4E). An adult chlamydial infection had no significant effect on any of these features of AAD (data not shown). Infected mice (neonatal and adult groups) that were not exposed to Ova (Cmu) had the same levels of inflammatory features as uninfected controls (Figures 4A–4E, Sal). Control groups receiving either SPG (instead of infection) or saline were not different in any features of AAD.

Effect of Infection on AHR

The development of AAD resulted in increased AHR to inhaled methacholine with significant increases in airway resistance that was associated with decreased dynamic compliance of the airways when compared with controls (Sal) ($P < 0.01$ or $P < 0.001$; Figure 5). This correlated with increased Th2 responses, eosinophil accumulation, and mucus hypersecretion in the lung. Chlamydial infection (Cmu) of both neonates ($P < 0.001$ vs. Sal or SPG) and adults ($P < 0.01$ for resistance and not significant for compliance) also caused a reduction in lung function later in life. This is in agreement with human studies,
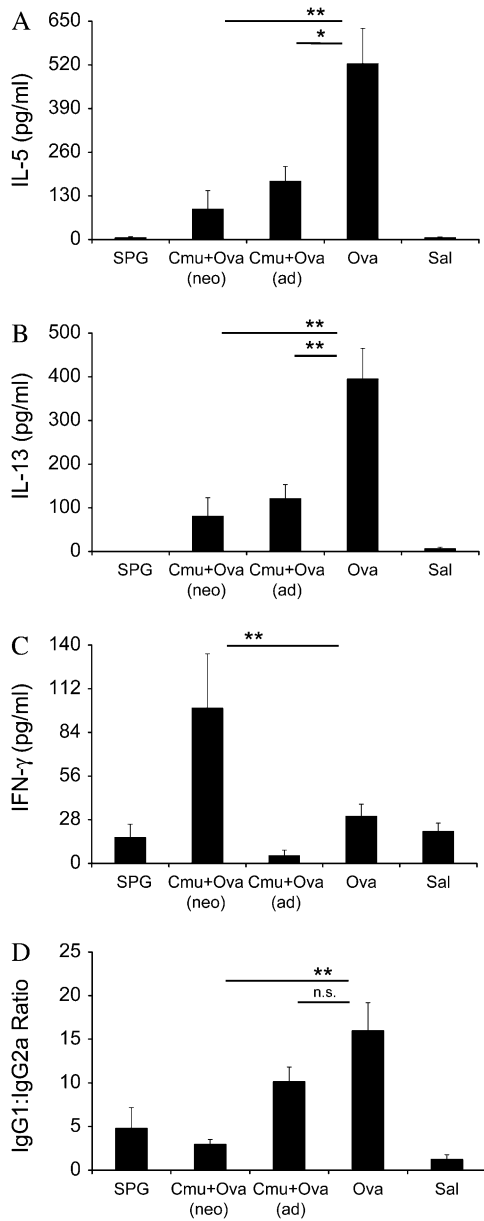
Figure 3. Ovalbumin (Ova)-specific lymph node T-cell cytokine production ([*A*] IL-5; [*B*] IL-13; [*C*] IFN-γ) and serum antibodies (IgG1:IgG2a ratios) in mice receiving *C. muridarum* infection and intraperitoneal sensitization to Ova (Cmu/Ova) compared with Ova or vehicle (sucrose–phosphate–glutamate buffer [SPG] or saline [Sal]) groups. n = 4 data points from combined lymph nodes from two mice per point in duplicate. Significant differences between Cmu/Ova and Ova are shown as *$P < 0.05$, **$P < 0.01$. n.s. = not significant.

Figure 4. Pulmonary inflammatory cells ([*A*] eosinophils in bronchoalveolar lavage fluid [BALF]; [*B*] eosinophils in tissue; [*C*] neutrophils in BALF), (*D*) mucus-secreting cells around airways, and (*E*) histopathology score in neonatal groups infected with *C. muridarum* and intraperitoneally sensitized (Cmu/Ova) as adults compared with infection (Cmu), ovalbumin (Ova), and vehicle (sucrose–phosphate–glutamate buffer [SPG] or saline [Sal]) groups. Significant differences between Cmu/Ova and Ova are shown as *$P < 0.05$, **$P < 0.01$, ***$P \leqslant 0.001$. Significant differences between Cmu and Cmu/Ova are shown as #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$. BM = basement membrane; n.s. = not significant.

in which respiratory chlamydial infection in children was associated with a decrease in lung function (43). Neonatal but not adult chlamydial infection induced further alterations in lung function after exposure of the airways to Ova, with significant deleterious alterations in airway resistance and compliance ($P < 0.01$ and $P < 0.05$, respectively, Cmu/Ova vs. Ova; Figure 5). The alterations in lung function observed in Cmu/Ova groups was not significantly different from Cmu groups. However, it is likely that the additional alterations in the Cmu/Ova group are significant but that the AHR had reached its maximal response (the same
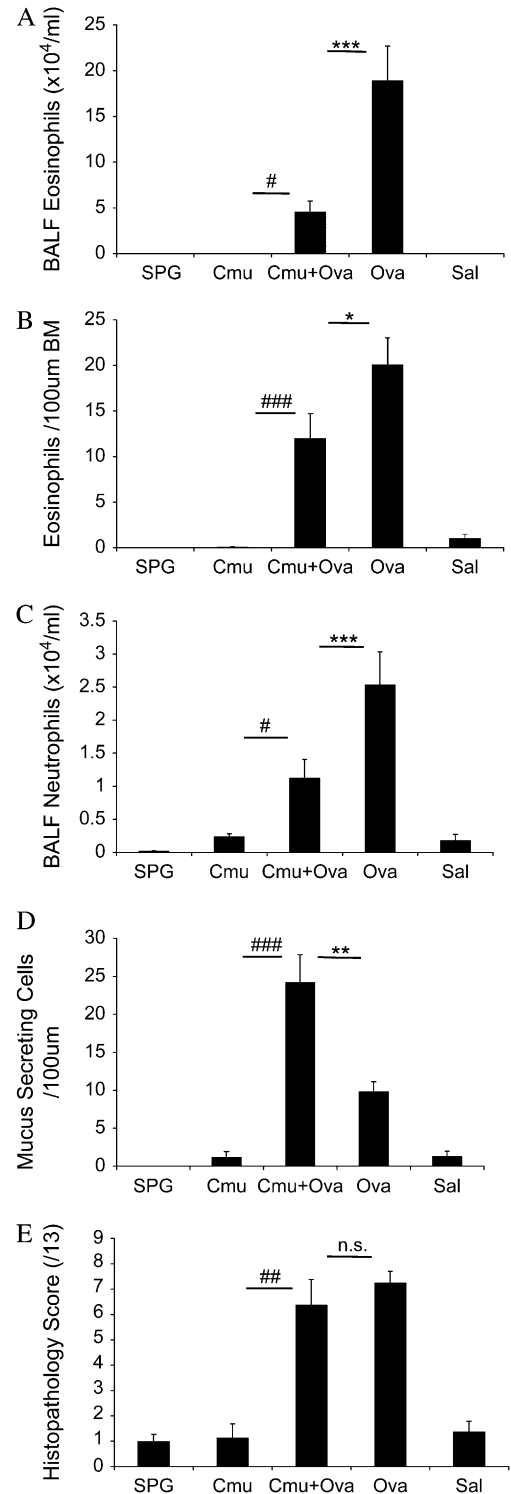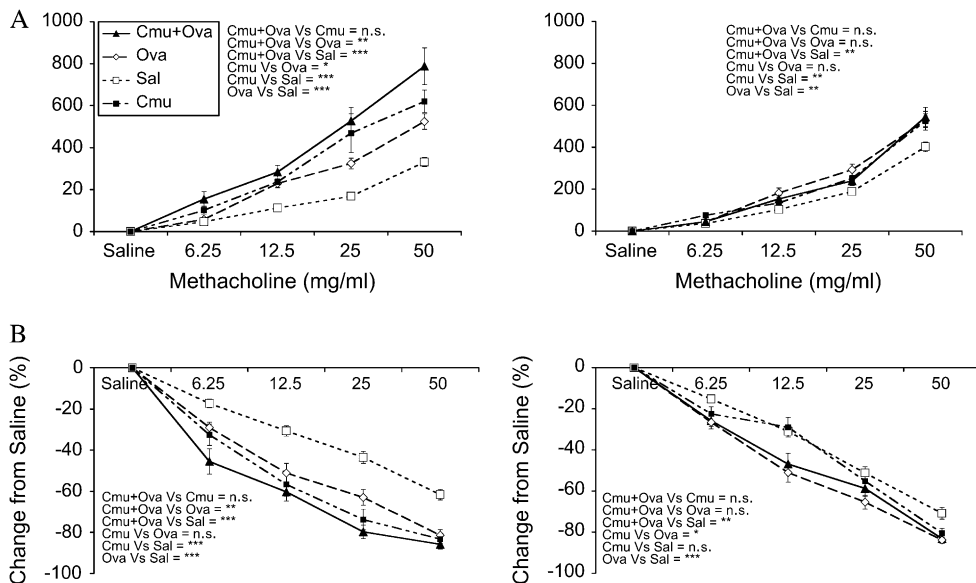
Figure 5. Airway hyperresponsiveness, measured as airway resistance (*A*) and dynamic compliance (*B*), after *C. muridarum* infection and intraperitoneal induction of allergic airway disease (Cmu/Ova), compared with infection (Cmu), ovalbumin (Ova), and vehicle (saline [Sal]) groups. Significant differences between groups are shown as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. n.s. = not significant.

level as that achieved by the intranasal administration of recombinant IL-13 that induces maximal AHR; data not shown). Airway reactivity of control groups receiving either SPG (vehicle for infection) or Sal was not significantly different or enhanced.

Duration of Effects of Infection on AAD

Neonatal infection led to prolonged expression of AAD after cessation of antigen challenge. Experiments with infection in the first day of life were repeated but the outcomes of AAD were examined 4 days after the final Ova challenge. In uninfected groups with AAD (Ova) IL-5, IL-13 (data not shown), and IFN-γ release from Ova-stimulated T cells, eosinophil (BALF and tissue) and neutrophil (BALF) influx into the lung, and AHR all subsided significantly (Figure 6) compared with 1 day after the final Ova challenge (Figures 3–5). However, in groups with neonatal chlamydial infection before sensitization (Cmu/Ova), increased levels of IL-5 and IFN-γ, eosinophil and neutrophil influx into the lung, and AHR were maintained (Figure 6).

DISCUSSION

In this investigation we have demonstrated that early-life chlamydial lung infection does not protect against the development of AAD in later life. Instead, early-life infection exacerbated the development of hallmark features of disease, which included mucus production and AHR. Notably, neonatal infection suppressed and skewed T-cell responses to Ova in adulthood from a normally polarized robust Th2 response to a mixed Th1/Th2 (but IFN-γ–dominated) phenotype. Infection resulted in the decreased production of IL-5 and IL-13 and increased secretion of IFN-γ from Ova-specific T cells. The skew toward a more dominant Th1 response was also reflected by a decrease in Ova-specific IgG1:IgG2a serum antibody ratios. Although early-life infection suppressed eosinophil accumulation in the airways, infiltrates were still a marked feature of Ova-induced inflammation in adulthood. Importantly, the generation of the mixed Ova-specific T-cell response and reduction in eosinophils was not protective against the development of disease (mucus hypersecretion and AHR). These effects on T-cell polarization and development of disease were age dependent because there were no differences in the expression of AAD after adult infection.

Moreover, infection prolonged the expression of AAD and these effects were restricted to early-life infection.

The current paradigm associated with the hygiene hypothesis is that early-life infections with microbes that stimulate Th1 immunity are required to modulate the Th2-dominated neonatal phenotype and enable the immune system to develop a balanced helper T–cell repertoire that protects against the development of allergy (44). Although chlamydial infections initiate and are cleared by Th1-mediated immune responses, clinical studies link chlamydial lung infection with the development of asthma in children. Indeed, an important study showed that 34 to 40% of children with asthma were infected in the blood and lung with *Chlamydia* and that atopy was strongly associated with infection (26). Therefore, the question arises: How can a Th1-inducing chlamydial infection predispose to the development of asthma?

Two hypotheses have been proposed to explain the association between Th1-inducing infections and asthma (8). Neonatal responses to infection are highly polarized toward Th2 immunity (reviewed by Hansbro and coworkers [8]) and it is, therefore, possible that a chlamydial infection in neonates reinforces rather than suppresses this response, which leads to aberrant Th2 responses to the infection. This may cause the immune system to mature with a more allergic phenotype (reviewed by Gern and Lemanske [45]) that fails to clear the bacteria, resulting in persistent infection and increasing the severity of Th2-type inflammatory responses to environmental antigens. This in turn may promote asthma in susceptible individuals. An alternative explanation is that Th1-inducing infections may cause a generalized inflammation of the airways that leads to the exacerbation of allergen-induced inflammation and asthma (9, 46).

We used novel models of neonatal and adult chlamydial lung infection and an established model of AAD (37) to investigate the association between early-life infection and the development of allergic inflammation of the lung in later life. *C. pneumoniae* lung infection in humans is generally mild or subclinical. Our models use infection with *C. muridarum*, which is a natural mouse pathogen and generates equivalent pathology in the mouse as *C. pneumoniae* in humans. Indeed, the time course of chlamydial growth and clearance and histopathological progression of disease closely resembles that observed in
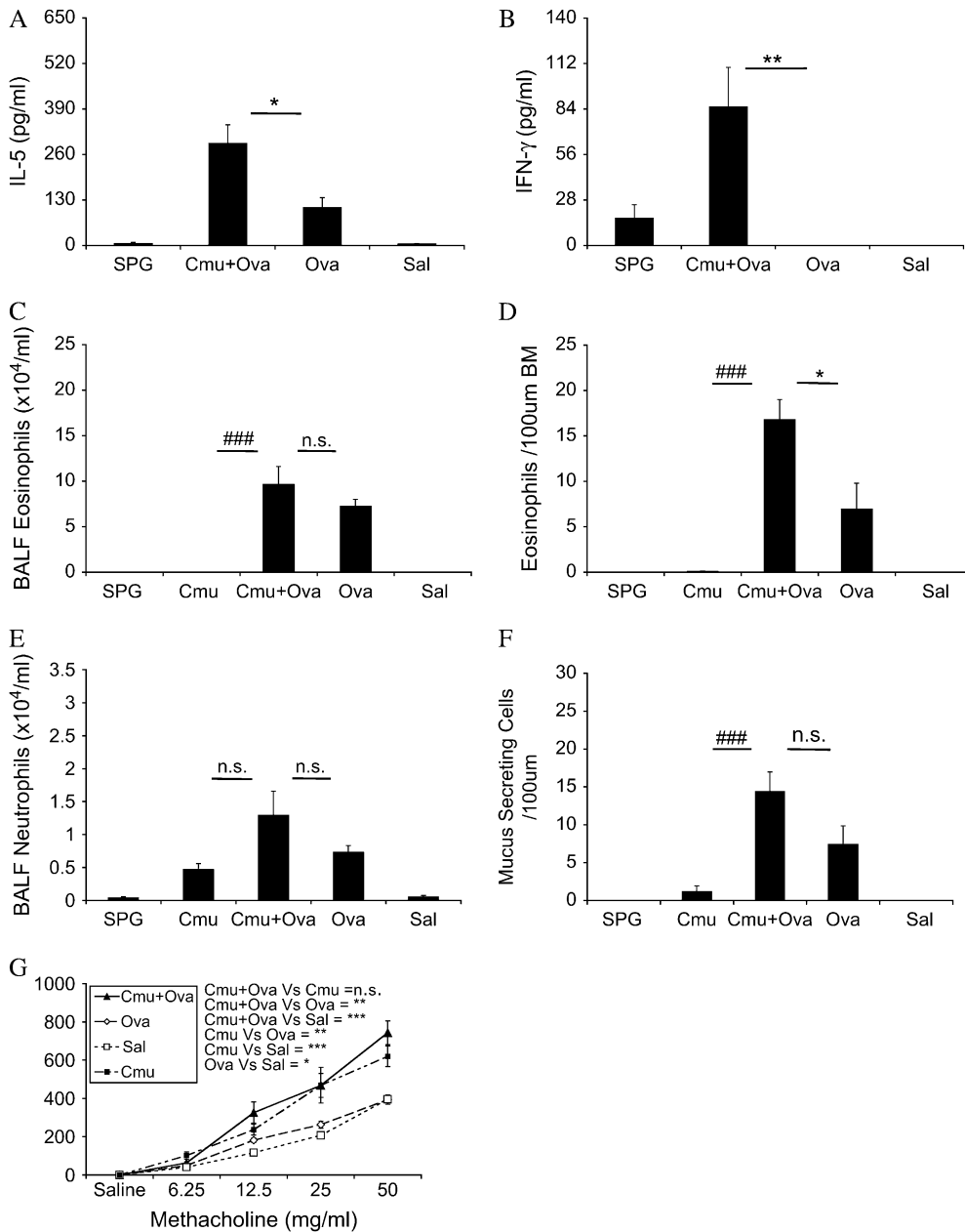
Figure 6. Ovalbumin (Ova)-specific lymph node T-cell cytokine production ([*A*] IL-5; [*B*] IFN-γ), pulmonary inflammatory cells ([*C*] eosinophils in bronchoalveolar lavage fluid [BALF]; [*D*] eosinophils in tissue; [*E*] neutrophils in BALF), (*F*) mucus-secreting cells around airways, and (*G*) airway hyperresponsiveness (airway resistance) in neonatal groups infected with *C. muridarum* and intraperitoneally sensitized (Cmu/Ova) adults 4 days after the final challenge compared with infection (Cmu), Ova, and vehicle (sucrose–phosphate–glutamate buffer [SPG] or saline [Sal]) groups. Significant differences between Cmu/Ova and Ova are shown as $*P < 0.05$, $**P < 0.01$. Significant differences between Cmu and Cmu/Ova are shown as $###P < 0.001$. Significant differences in airway hyperresponsiveness (*G*) are shown as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. n.s. = not significant.

human *C. pneumoniae* infection (32–36, 39, 47). The only clinical symptom observed in our investigation was a reduction in the rate of weight gain 2 to 4 weeks after infection compared with uninfected controls.

We found no evidence of chronic chlamydial infection (6 wk after infection) by real-time PCR or by tissue culture and neonatally infected mice responded with more of a Th1 phenotype to Ova exposure. Thus, our results demonstrate that infection early in life results in immune responses that clear the active infection and that infection per se does not increase Th2 responses to Ova. Remarkably, however, early-life infection substantially alters the way the adaptive immune system responds to unrelated antigens. In this regard neonatal infection resulted in the development of Ova-specific Th1 cells and Th1-skewed antibody responses (decreased IgG1:IgG2a ratio) after systemic Ova priming. Systemic exposure of Ova in Rehydragel (alum) according to the protocol used induces robust and highly polarized Th2 immune responses. Notably, although attenuated, the

propensity for the development of the Th2 response persisted, and T-cell–derived IL-5 and IL-13 and eosinophilia were still observed. Even though Th2 responses were present the mixed phenotype suppressed the development of eosinophilic inflammation (most likely through a reduction in IL-5 and IL-13 release from T cells). IL-5 plays a key role in the differentiation, maturation, recruitment, and activation of eosinophils (reviewed by Kay and coworkers [48]) and IL-13 induces the production of eotaxins (49, 50). Although these aspects of allergy are reduced, early-life infection resulted in the exacerbation of mucus production and AHR. Although infection alone led to decreased lung function, neonatal infection resulted in a further increase in Ova-induced AHR.

Thus, the outcome of early-life infection is the development of an inflammatory disease of the airways that has typical features of asthma (eosinophil influx, mucus-secreting cell expression, and AHR) in the presence of an antigen-specific IFN-γ–dominated response in association with Th2 cell responses. Th1 cells do not

induce these features of disease alone as adoptive transfer studies have shown that airway infiltrates are composed predominantly of mononuclear cells (lymphocytes and monocytes and a slight increase in eosinophils) and a reduction in mucus production. Importantly, this Th1-induced inflammation and disease is not associated with the induction of AHR (51, 52). By contrast, our results demonstrate that the Th1 and Th2 cytokines produced by T cells cooperate to induce key pathogenic features of asthma (53). This cooperative mechanism between Th1 and Th2 cytokines has also been observed in chronic models of asthma and in adoptive transfer models of disease with IFN-γ/IL-5–secreting inflammatory helper T (Thi) cells (41, 53). Our observations highlight the complex nature of the interaction between infection, T cell programming, and developmental age, which underpins the onset of this cooperative mechanism.

Furthermore, we also demonstrated that early-life infection induced the persistence of the important components of disease. Indeed, although the features of AAD subside in allergic groups soon after the cessation of antigen exposure in groups that receive neonatal infection, the features of disease are maintained.

The first 24 hours after birth is an immune-privileged period. Therefore to determine whether the observed effects were restricted to the immune-privileged phase experiments were repeated with infection (400 inclusion-forming units) 1 week after birth. We observed that infection again enhanced the major features of AAD but with a different inflammatory profile compared with infection within the first 24 hours of birth. There was a significant increase in IL-5 and IL-13 but no change in IFN-γ release from Ova-specific T cells. This was accompanied by significant elevations in eosinophil but not neutrophil influx into the BALF and lung tissue, mucus hypersecretion, and AHR. Therefore, chlamydial infection during early life enhances AAD in later life *per se*. Future work will determine the causes of the age-related differences between these two groups.

Taken together, our results suggest that the induction of inflammatory changes in the developing lung by early-life chlamydial infection may set in motion processes that promote Ova-specific T-cell responses of a mixed phenotype that lead to histopathological and functional abnormalities (mucus secretion, AHR) later in life. Notably, this inflammatory process may be directly relevant to asthma as this mixed inflammatory response has been detected in patients with asthma, who often have high IFN-γ responses in combination with elevated Th2 responses (54, 55). It is also known that processes associated with some early-life infections, such as viral infections (reviewed by Gern and colleagues [56]), may have long-term adverse effects on lung function including chronic airway inflammation, remodeling, alveolarization, and epithelial dysfunction that lead to asthma.

Although studies have shown that neonatal viral infection or antigen exposure can alter the nature of subsequent responses to antigens and influence the development of immune and physiological systems later in life (57, 58), this is the first report of how early-life bacterial infection can exacerbate the development of AAD in adulthood. Previous studies with chlamydial lung infections in adult mice produced results different from those described in our study. They showed that airway eosinophilia in response to ragweed (a Th2-inducing antigen) was significantly reduced by previous infection in adulthood (59). Interestingly, these authors demonstrated that dendritic cells expressing Toll-like receptor 9 from *Chlamydia*-infected but not uninfected adult mice modulated responses to Ova away from the allergic phenotype (local and systemic eosinophilia) by reducing IL-5 and IL-13 expression from T cells (60). Thus these infection studies in the adult were interpreted as providing protection against the expression of AAD, rather than exacerbating disease. By contrast, we showed that there was no effect of an adult infection on eosinophil numbers in the airways or on IL-5 or IL-13 release from T cells. Furthermore, these investigations did not examine the effect of infection on mucus production or lung function and were performed largely in C57BL/6 mice, which are more resistant to chlamydial infection than are BALB/c mice. Interestingly, the suppression of allergic disease in the ragweed model was thought to result in a switch from Th2 to Th1 cytokines, but this was not reflected in alterations in specific or total antibodies.

In our studies, the induction of both Th1 and Th2 responses occurs in a model of AAD that is based on systemic priming with a strong Th2-inducing antigen and adjuvant (Ova in alum), which normally results in highly polarized Th2 responses. Thus, the effect of early-life chlamydial infection on immunity and subsequent T-cell programming is substantial.

Our observations may be further investigated to identify pivotal immune processes in the neonate that regulate how T cells respond to antigens later in life, which may provide insights into therapeutic strategies to prevent the development of asthma. The mechanisms of how this occurs may be generally important in the development in humans of chronic inflammatory lung diseases that are initiated by early-life infections.

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