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Allergic Lung Inflammation Reduces Tissue Invasion and Enhances Survival from Pulmonary Pneumococcal Infection in Mice, Which Correlates with Increased Expression of Transforming Growth Factor β1 and SiglecF^{low} Alveolar Macrophages

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Asthma is generally thought to confer an increased risk for invasive pneumococcal disease (IPD) in humans. However, recent reports suggest that mortality rates from IPD are unaffected in patients with asthma and that chronic obstructive pulmonary disease (COPD), a condition similar to asthma, protects against the development of complicated pneumonia. To clarify the effects of asthma on the subsequent susceptibility to pneumococcal infection, ovalbumin (OVA)-induced allergic lung inflammation (ALI) was induced in mice followed by intranasal infection with A66.1 serotype 3 *Streptococcus pneumoniae*. Surprisingly, mice with ALI were significantly more resistant to lethal infection than non-ALI mice. The heightened resistance observed following ALI correlated with enhanced early clearance of pneumococci from the lung, decreased bacterial invasion from the airway into the lung tissue, a blunted inflammatory cytokine and neutrophil response to infection, and enhanced expression of transforming growth factor β1 (TGF-β1). Neutrophil depletion prior to infection had no effect on enhanced early bacterial clearance or resistance to IPD in mice with ALI. Although eosinophils recruited into the lung during ALI appeared to be capable of phagocytizing bacteria, neutralization of interleukin-5 (IL-5) to inhibit eosinophil recruitment likewise had no effect on early clearance or survival following infection. However, enhanced resistance was associated with an increase in levels of clodronate-sensitive, phagocytic SiglecF^{low} alveolar macrophages within the airways following ALI. These findings suggest that, while the risk of developing IPD may actually be decreased in patients with acute asthma, additional clinical data are needed to better understand the risk of IPD in patients with different asthma phenotypes.

Streptococcus pneumoniae is a Gram-positive, extracellular bacterium commonly found in the upper respiratory tract and is recognized as the leading cause of community-acquired pneumonia worldwide (1). *S. pneumoniae* infections can range from asymptomatic carriage to more-severe outcomes such as otitis media, pneumonia, and pneumococcal meningitis. The capacity for invasion from a carrier state is directly related to the capsule polysaccharide serotype. Over 90 serotypes are known to exist (2), which complicates the clinical effectiveness of vaccination and emphasizes the importance of developing a better understanding of the immunological response to acute pneumococcal infections in the lung.

Mortality following invasive pneumococcal infections is significantly higher among at-risk populations, such as the elderly or individuals with underlying medical conditions (3). Asthma has recently been identified as a significant risk factor for invasive pneumococcal disease (4–6). This increased risk has also been extended to other atopic conditions such as allergic rhinitis and hay fever (7), and the Advisory Committee on Immunization Practices has included individuals with asthma among those indicated for pneumococcal polysaccharide vaccination (8). Considering that asthma now affects over 8% of the population in the United States and the prevalence appears to be increasing (9), there is a pressing need for further knowledge about pneumococcal infections in the context of asthma.

Alveolar macrophages (AMs) are the first cells that respond to pneumococcal infection within the lung, and the phagocytic capacity of these cells is a critical determinant of disease outcome (10). Subsequent intense inflammation highlighted by neutrophil recruitment occurs if the alveolar macrophage defenses are overwhelmed and fail to control bacterial outgrowth (11). A decreased ability to clear bacteria from the lung in individuals with asthma has been postulated as the main reason for an increased risk of pneumococcal infection in this population (6). Unlike chronic obstructive pulmonary disease (COPD), which has been shown to severely impair lung phagocytic function (12, 13), decreased clearance of only *Staphylococcus aureus* and *Haemophilus influenzae* has been reported in patients with severe asthma (14) and in children with poorly controlled asthma (15). We have now examined in detail the influence of allergic lung inflammation (ALI) on clearance of pneumococci from the lung and survival from pneumococcal pneumonia. Our results show that ALI protects against

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pulmonary pneumococcal infection by significantly enhancing bacterial clearance and reducing inflammation. This was associated with an increase in SiglecF^{low} macrophage accumulation within the airways and lung following ALI induction.

MATERIALS AND METHODS

Mice. Female BALB/c mice (5 to 6 weeks old) were purchased from Charles River Laboratories through a contract with the National Cancer Institute. Mice were housed in the Animal Resources Facility at Albany Medical College, and all procedures were approved by the Institutional Animal Care and Use Committee.

ALI. To induce ALI with ovalbumin (OVA), mice were sensitized by two weekly intraperitoneal (i.p.) injections of 10 μg of OVA (Sigma) combined with 4 mg of aluminum hydroxide (General Chemical) and 100 μl of phosphate-buffered saline (PBS). One week later, the mice were lightly anesthetized with isoflurane and intranasally (i.n.) challenged with 100 μg of OVA–50 μl PBS for 5 days, as previously described (16, 17). Control mice were sensitized by i.p. OVA injections but challenged i.n. with PBS only.

Pneumococcal infection model. To induce pneumococcal infection, mice were anesthetized by i.p. injection of 100 μl of ketamine (1 mg/ml)–xylazine (20 mg/ml)–PBS and were then inoculated i.n. with 10^5 CFU of *S. pneumoniae* strain A66.1–40 μl PBS. The animals were monitored daily for survival. To assess bacterial burden in the bronchoalveolar lavage (BAL) fluid and lung tissue, mice were sacrificed at 8, 24, or 48 h postinfection by i.p. injection of sodium pentobarbital per the instructions of the manufacturer (Zoetis, Inc.). BAL fluid was collected by performing lavage 3 times with 1 ml sterile PBS. Lung tissue homogenates were prepared by bead disruption. Samples were then centrifuged, and serial dilutions of supernatants were plated on blood agar plates. CFU were quantified following overnight incubation at 37°C.

Cytokine measurements. Following euthanasia, a cannula was inserted into the trachea and the lungs were subjected to lavage 3 times with 1 ml cold PBS. The recovered fluid was centrifuged, and the supernatant was divided into aliquots and stored at −80°C. Total transforming growth factor β1 (TGF-β1) was measured in the BAL fluid using a Bio-Plex Pro multiplexed assay (Bio-Rad) per the manufacturer's instructions. For lung tissue cytokine measurement, lung homogenates were prepared with silica beads and the supernatants were tested using a cytometric bead array (BD Biosciences) per the manufacturer's instructions.

Flow cytometry. For flow cytometric analysis, BAL fluid and lung cells were incubated with fixable viability dye (eBioscience), and Fc receptors were blocked with anti-mouse FcγII/III receptor monoclonal antibody (MAb) 2.4G2 for 20 min at 4°C, washed, and then incubated with cell subset-specific MAbs for 25 min at 4°C. The following MAbs were used: phycoerythrin (PE)-Cy7-conjugated anti-CD11c (clone HL3; BD Biosciences), allophycocyanin (APC)-conjugated anti-CD11c (clone HL3; BD Biosciences), PE-Texas Red-conjugated anti-CD11b (clone M1/70; Invitrogen), peridinin chlorophyll protein (PerCP)-Cy5.5-conjugated CD11b (clone M1/70; BD Pharmingen), fluorescein isothiocyanate (FITC)-conjugated anti-Ly6G (clone 1A8; BD Pharmingen), PE-Cy7-conjugated anti-Ly6G (clone 1A8; Biolegend), and Alexa Fluor 647-conjugated anti-SiglecF (clone E50-2440; BD Pharmingen). Stained cells were analyzed using a FACSCanto flow cytometer and FlowJo software.

***In vivo* cell depletions following OVA-induced ALI.** To block eosinophil recruitment into the pulmonary tract, 100 μg of anti-interleukin-5 (anti-IL-5) MAb clone TRFK5 (BioXCell) was administered i.p. daily for five consecutive days simultaneously with i.n. OVA challenge and on days 3 and 7 after the final OVA challenge (>95% depletion). AMs were depleted by i.n. administration of 50 μl of liposome-encapsulated clodronate (FormuMax) to anesthetized mice (>75% depletion) 2 days prior to infection with *S. pneumoniae*. Neutrophils were depleted by i.p. treatment with 500 μg Ly6G MAb clone 1A8 (BioXCell) 3 days before infection and with 250 μg 2 days after infection (>90% depletion). Depletion efficiency was confirmed by flow cytometry for all experiments.

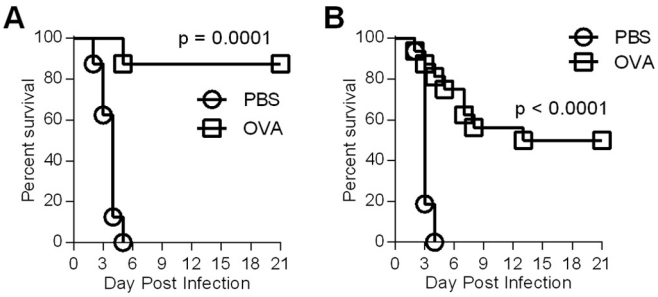

FIG 1 ALI protects against pulmonary pneumococcal infections. (A) Following OVA sensitization and challenge to induce ALI, mice were infected i.n. with 1×10^5 CFU of *S. pneumoniae* A66.1 and survival was monitored for 21 days (eight mice per group). The data shown are representative of the results of three independent experiments. (B) Following sensitization and challenge with OVA to induce ALI, mice were infected i.n. with 4×10^5 CFU of *S. pneumoniae* A66.1 and survival was monitored for 21 days (16 mice per group). The data represent combined results from two independent experiments.

Statistical analysis. The data are expressed as means ± standard deviations (SD); analysis of variance (ANOVA) (for multiple comparisons) and Student's *t* test (to compare two samples) were used for statistical analyses of bacterial clearance, cytokine production, and cell expression. Survival analyses were performed using the Mantel-Cox log rank test. Statistical procedures were performed using GraphPad Prism 6 software. *P* values of <0.05 were considered statistically significant.

RESULTS

Allergic lung inflammation enhances resistance to invasive pneumococcal infection. To assess the impact of acute ALI on susceptibility to pulmonary pneumococcal infection, an established model of OVA-induced ALI was used, followed at various intervals by i.n. infection with 1×10^5 CFU of serotype 3 *S. pneumoniae* strain A66.1. A peak level of survival from pneumococcal infection was observed 10 days after the final OVA challenge (see Fig. S1 in the supplemental material) and waned thereafter (data not shown). Thus, we focused on this time point for all subsequent experiments and again observed that mice with ALI were highly resistant to pneumococcal infection (80% survival over 21 days compared to 100% mortality within 5 days in non-ALI controls) (Fig. 1A). Increased resistance at an even higher pneumococcal challenge dose of 4×10^5 CFU was also observed (Fig. 1B). Use of a house dust mite (HDM)-induced ALI model (18) also resulted in enhanced resistance to pneumococcal pneumonia (see Fig. S2), which confirmed the finding that ALI enhances resistance to pulmonary pneumococcal infections in mice.

The inflammatory response to pneumococcal infection was also examined in the lungs of mice with ALI as an indication of pathology. Following infection, non-ALI mice produced significant levels of lung tumor necrosis factor alpha (TNF-α), IL-6, and gamma interferon (IFN-γ), while mice with ALI produced virtually undetectable levels of these cytokines (Fig. 2A). This reduced proinflammatory cytokine production was accompanied by increased production of the anti-inflammatory cytokine TGF-β1 in the airways following infection (Fig. 2B). Significantly reduced neutrophil recruitment to the airways and lung tissue was also observed following infection (Fig. 2C), with baseline neutrophil numbers remaining essentially unchanged following bacterial challenge. Thus, mice recovering from ALI have increased survival

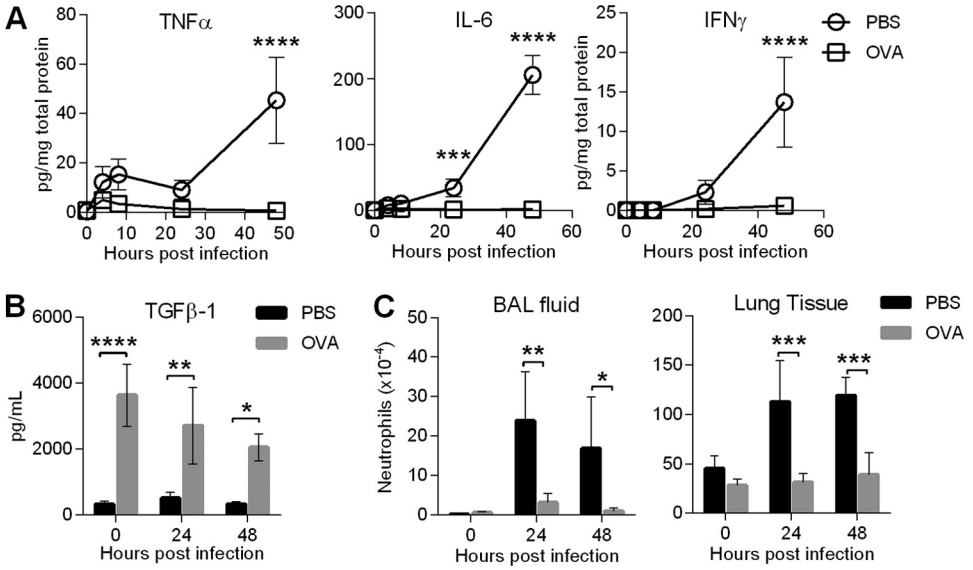

FIG 2 ALI constrains infection-induced inflammation. (A) Mice were infected i.n. with 1×10^5 CFU of *S. pneumoniae* A66.1, lung tissue homogenates were prepared at the indicated time points, and cytokine levels were measured by cytometric bead array. The data were obtained from the results of one experiment with four mice/group and are shown as means ± SD. ***, $P < 0.001$; ****, $P < 0.0001$ (by two-way ANOVA). (B) Total TGF-β1 levels were measured by Bioplex in BAL fluid before and at the indicated times following i.n. infection with 1×10^5 CFU of *S. pneumoniae* A66.1 in PBS- or OVA-challenged mice. The data represent means ± SD of the results of one of two independent experiments (four mice per group). *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$ (by two-way ANOVA). (C) Quantification of total $CD11c^-$ $CD11b^+$ $Ly6G^+$ $SiglecF^-$ neutrophils in the airway (left panel) and lung tissue (right panel) before and following i.n. infection with 1×10^5 CFU of *S. pneumoniae* A66.1. Data represent means ± SD of the results of one of two independent experiments (four mice per group). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (by two-way ANOVA).

and reduced immunopathology following lethal pneumococcal challenge.

Effect of ALI on lung bacterial clearance. The clearance of bacteria from the lung following ALI was next examined. Mice with ALI were found to clear significantly more bacteria from the lung within 8 h than non-ALI mice across a wide range of infectious challenges (Fig. 3A). To assess the importance of this bacterial clearance, ALI mice and non-ALI controls were infected with 1×10^5 and 2.5×10^4 CFU, respectively, which were concentrations that corresponded to equivalent bacterial burdens observed in both groups 8 h after infection (filled symbols in Fig. 3A). This resulted in comparable mortality rates in these groups (Fig. 3B), suggesting that the increased clearance observed in mice following ALI is a critical event that promotes survival of an otherwise lethal pulmonary infection. The overall kinetics of bacterial clearance from the airways and lung tissue were also examined. Mice with ALI had significantly reduced levels of bacteria in the airways (Fig. 3C, left) and lung tissue (Fig. 3C, right) following infection. While bacterial outgrowth and increased invasion from the airways to the lung parenchyma in non-ALI mice occurred between 24 and 48 h postinfection, mice with ALI had consistently reduced bacterial burdens in the airways and lung, with most ALI mice clearing the infection by 72 h postinfection (data not shown). Taken together, these results demonstrate that ALI enhances protection against pulmonary pneumococcal infection by augmenting bacterial clearance and controlling outgrowth in the respiratory tract.

AM depletion abrogates enhanced survival following ALI. To identify the cell populations that were responsible for enhanced bacterial clearance following ALI, mice were inoculated with heat-killed and FITC-labeled *S. pneumoniae* A66.1 1 h before collection of BAL fluid cells for flow cytometric analysis. Using this ap-

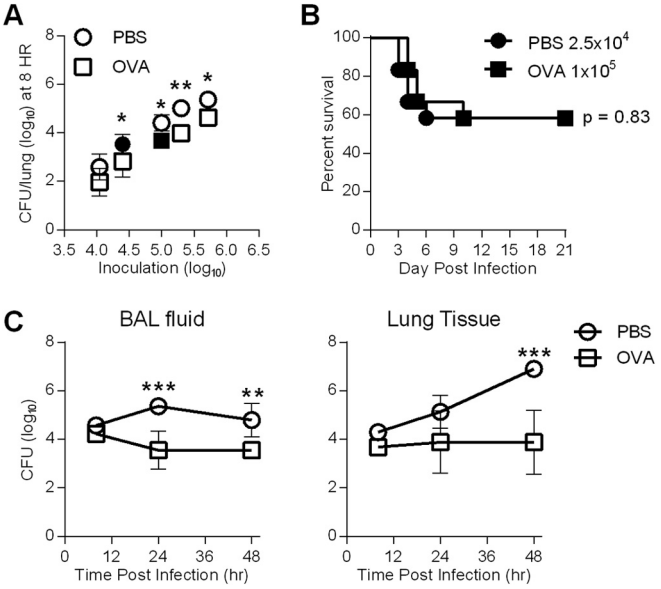

FIG 3 ALI enhances early bacterial clearance and reduces tissue invasion. (A) Mice were infected i.n. with the indicated doses of *S. pneumoniae* A66.1 following ALI induction. Lung tissue was collected after 8 h and homogenized, and 10-fold serial dilutions were plated on blood agar plates. The data represent means ± SD and are combined from the results of five independent experiments (five mice per group). *, $P < 0.05$; **, $P < 0.01$ (by two-way ANOVA). HR, hours. (B) Survival was monitored after infection with doses that resulted in comparable CFU counts after 8 h of infection (filled symbols in panel A) (12 mice per group). The data are from the combined results of two independent experiments. (C) Following infection with 1×10^5 CFU of *S. pneumoniae* A66.1, mice were sacrificed at the indicated times and bacterial levels were determined in BAL fluid and lung tissues. The data represent means ± SD of the results of one of two independent experiments (four mice per group). **, $P < 0.01$; ***, $P < 0.001$ (by two-way ANOVA).

proach, we observed that the total number of CD11b^{med} CD11c^{+} AMs was increased approximately 2-fold in ALI mice compared to non-ALI mice (see Fig. S3B, left panel, in the supplemental material); however, there was no increase in the number of AMs that bound to FITC-labeled pneumococci at this early time point (see Fig. S3B, right panel). Mice with ALI were also found to have an increased number of CD11b^{hi} CD11c^{-} Ly6G^{-} cells in the airways (see Fig. S3C, left panel) that were able to bind to the FITC-labeled pneumococci (see Fig. S2C, right panel). Utilizing imaging flow cytometry, we then observed that the recruited CD11b^{hi} CD11c^{-} cells also expressed SiglecF (see Fig. S3D), identifying them as eosinophils (19), and that these eosinophils phagocytosed PKH-26-labeled pneumococci, as shown by merged bright-field and internalized *S. pneumoniae* images (see Fig. S2D, right column).

The functional significance of pneumococcal phagocytosis by eosinophils was next investigated by treating mice with anti-IL-5 MAb, which has been shown to block recruitment of eosinophils to the lung (20). Blocking IL-5 concurrent with allergen challenge significantly reduced eosinophil recruitment to the airways (see Fig. S4B in the supplemental material) and lung tissue (see Fig. S4C), while having no effect on the number of CD11c^{+} cells or neutrophils in either compartment (see Fig. S4B and C). However, blocking eosinophil recruitment in mice with ALI had no impact on the enhanced 8-h clearance (Fig. 4A) or on the enhanced survival observed after pneumococcal infection (Fig. 4B). Although neutrophils have been shown to be important following pulmonary bacterial infections (21–24), we observed no significant difference in 8-h bacterial burden or mortality following infection of neutrophil-depleted ALI mice (Fig. 4C and D), demonstrating that neutrophils also play a limited role in combating pneumococcal infection in mice with ALI.

Since depletion of eosinophils and neutrophils had limited effects on survival following pneumococcal infection in mice with ALI, the potential role of AMs was next investigated using i.n. treatment with liposomal clodronate, which has been shown to selectively deplete AMs (25). Depletion of AMs reversed the enhanced 8-h clearance of bacteria (Fig. 4E) and completely abrogated the increased survival observed in ALI mice (Fig. 4F). AM-depleted ALI mice were highly susceptible to an infectious challenge with as little as 250 CFU (data not shown), which suggests that AMs are the main effectors responsible for resistance to pulmonary pneumococcal infection in mice with ALI.

AM populations in mice with ALI. Considering that AM levels were increased in mice following ALI and were also critical for increased resistance to pneumococcal infection, the AM cell population present in ALI mice was further characterized. Recently, a new SiglecF^{low} AM subset has been described during the fibrotic phase of acute lung injury (19) and following allergen challenge in asthmatics (26). This AM population also expresses high levels of scavenger receptors CD36 (19) and MARCO (26), both of which can facilitate phagocytosis of unopsonized pneumococci (27–29). It was found that, following OVA-induced ALI, approximately one-third of the AM pool consisted of CD11c^{+} SiglecF^{low} cells, while these cells were absent from the lungs of non-ALI controls (Fig. 5A). A similar population of SiglecF^{low} AMs was observed in the airways of HDM-treated mice (see Fig. S5 in the supplemental material). The SiglecF^{low} population was also observed to be sensitive to i.n. clodronate treatment, highlighting its phagocytic capacity (Fig. 5B). The numbers of SiglecF^{low} AMs were significantly increased in ALI mice, while total levels of traditional SiglecF^{hi} AMs (Fig. 5C) and of those positive for FITC-labeled fluorescent beads were equivalent for mice with or without ALI (Fig. 5D). All of the SiglecF^{low} AMs appeared capable of phagocytizing FITC-labeled latex beads, with a median fluorescent intensity ± SD of $2.6 \times 10^4 \pm 4.5 \times 10^3$ compared to $4.1 \times 10^4 \pm 1 \times 10^4$ for traditional SiglecF^{hi} AMs (based on 5 mice/group; see Fig. 5E for representative staining results). Overall, these results suggest that this newly recruited population of SiglecF^{low} AMs contributed to the enhanced clearance of pneumococci from the lungs during recovery from ALI.

DISCUSSION

In the present study, we found that induction of ALI protected mice against an otherwise lethal pulmonary pneumococcal infection. Despite recent reports suggesting an association between asthma and invasive pneumococcal infection (4–6), mice with ALI were able to clear significantly more pneumococci from the pulmonary tract after infection and this enhanced clearance correlated with improved survival, diminished neutrophil recruitment, and reduced expression of inflammatory cytokines in the lung. The effect of ALI on resistance to pneumococcal infection was confirmed using an HDM-induced ALI model. We also detected a new population of phagocytic SiglecF^{low} AMs that were present following ALI induction, suggesting that these cells were responsible for the enhanced resistance of mice with ALI.

It was found that, after pulmonary infection with serotype 3 A66.1 *S. pneumoniae*, nearly all mice that had been sensitized and challenged i.n. with OVA 10 days earlier survived the bacterial challenge. On the other hand, all mice sensitized to OVA but challenged with PBS died within 5 days. Induction of resistance appeared to be most prominent 10 days after allergen challenge; animals infected at earlier time points following OVA challenge demonstrated significant and yet reduced levels of resistance. The reason for this reduced resistance at earlier time points is unknown but could have been due to high levels of proinflammatory cytokines remaining in the lungs shortly after ALI and/or a reduced level of phagocytic AMs. The impact of ALI on pulmonary bacterial infections was investigated previously utilizing multiple mouse models, with differing results. Employing *in vivo* bioluminescent imaging, it was found that ALI reduced the level of pneumococcal burden in the lung, although there was a trend toward enhanced bacterial invasiveness (30). In a pulmonary *Pseudomonas* infection model, it was reported that OVA sensitization and challenge inhibited antibacterial defenses (31). On the other hand, ALI decreased lung infection by *Klebsiella pneumoniae* (32), a respiratory pathogen with etiology similar to that of *S. pneumoniae*. OVA-induced ALI had no effect on the response to *S. pneumoniae* serotype 4 aerosol infection (33), although HDM-induced ALI increased lung bacterial levels due to diminished Toll-like receptor signaling (34). It is important that, in the latter study, the authors infected the animals with *S. pneumoniae* 3 days following the final HDM challenge, while we found that the height of ALI-induced resistance occurred 10 days following allergen challenge.

In our study, mice with ALI clearly had significantly better clearance of pneumococci from the airways and lungs, prompting us to examine the expression of phagocytic cell populations that might be impacted by ALI at these sites. Asthma is known to enhance eosinophil recruitment into the lung parenchyma, and we likewise observed an increase in pulmonary eosinophil numbers after OVA sensitization and challenge. Furthermore, the eosino-

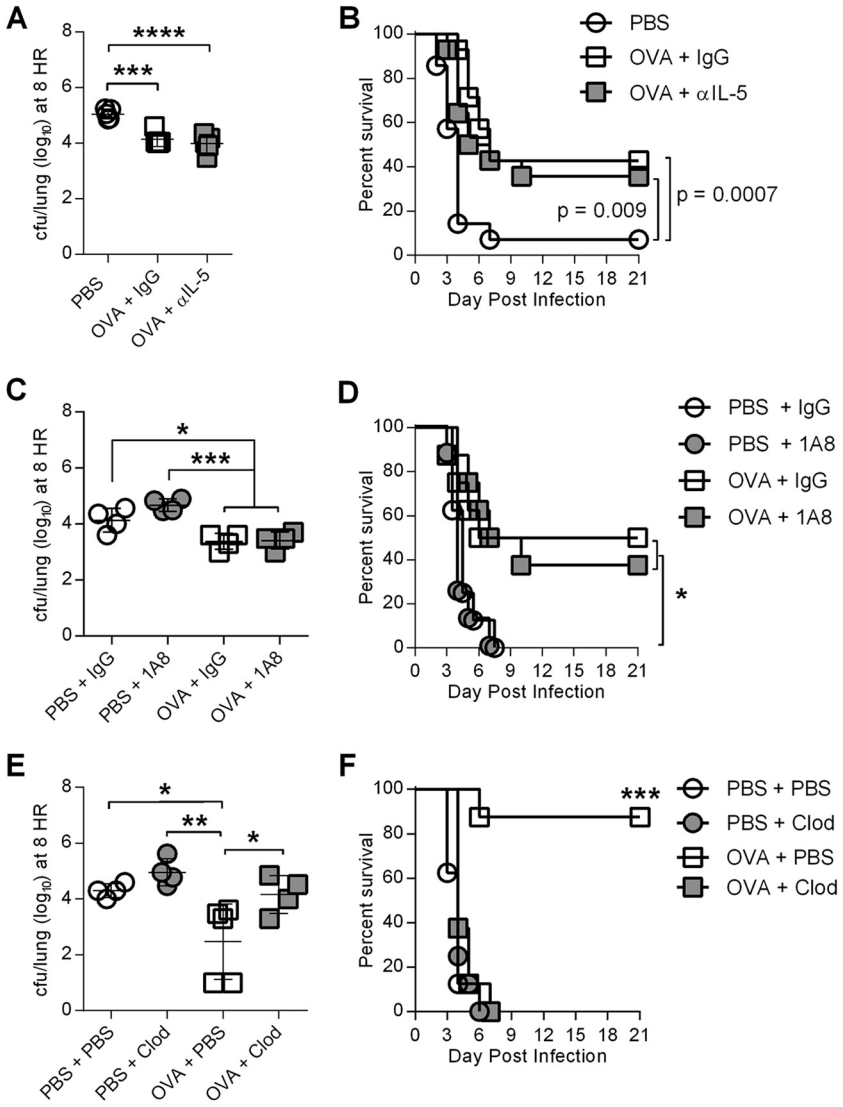

FIG 4 ALI-enhanced survival is abrogated in the absence of AMs. (A and B) PBS- or OVA-treated mice with or without i.p. injection of anti-IL-5 MAb or IgG isotype control during the OVA challenge were infected i.n. with 1×10^5 CFU of *S. pneumoniae* A66.1. (A) Lung tissue CFU counts were determined after 8 h. The data represent means ± SD of the results of one of two independent experiments (five mice per group). ***, $P < 0.001$; ****, $P < 0.0001$ (by two-way ANOVA). (B) Mortality was monitored for 21 days (14 mice per group). The data are from the combined results of two independent experiments. (C and D) PBS- or OVA-treated mice with or without i.p. injection of MAb 1A8 or IgG isotype control to deplete neutrophils were infected i.n. with 1×10^5 CFU of *S. pneumoniae* A66.1. (C) Lung tissue CFU counts were determined after 8 h. The data represent means ± SD of the results of one of three independent experiments (four mice per group). *, $P < 0.05$; ***, $P < 0.001$ (by two-way ANOVA). (D) Mortality was monitored for 21 days (eight mice per group) *, $P < 0.05$ (compared to PBS-treated groups). The data shown are from the results of one of three independent experiments. (E and F) PBS- or OVA-treated mice with or without previous i.n. inoculation of clodronate (Clod)-loaded liposomes to deplete AMs were infected i.n. with 1×10^5 CFU of *S. pneumoniae* A66.1. (E) Lung tissue CFU counts were determined after 8 h. The data were obtained from the results of one experiment performed with four or five mice per group and represent means ± SD. *, $P < 0.05$; **, $P < 0.01$ (by two-way ANOVA). (F) Mortality was monitored for 21 days (eight mice per group). ***, $P < 0.001$ (compared to all groups). The data represent results of one of two independent experiments.

phils appeared to be able to phagocytize pneumococci. Treatment with anti-IL-5 MAb prevented recruitment of these cells to the lung following ALI; however, such treatment did not influence survival following bacterial infection. Similarly, depletion of neutrophils had little effect on resistance of ALI mice to bacterial infection. We concluded from these results that eosinophils and neutrophils were not required for enhanced resistance to pneumococcal pneumonia following ALI. In contrast to our findings, increased resistance of mice to *Klebsiella pneumoniae* following ALI did require neutrophils (32), a finding that is consistent with a requirement of neutrophils for protection against pulmonary *K. pneumoniae* infections (35). For pneumococcal infection, on the other hand, neutrophil recruitment is thought to play a major role in control of bacterial colonization while having a limited impact during acute lung infections (36–38).

We found that ALI-mediated resistance to pneumococcal infection was highly dependent upon the presence of AMs. Interestingly, we observed an unusual accumulation of SiglecF^{low} AMs in the airways following allergen sensitization and challenge. These cells were able to bind to latex beads and were sensitive to lipo-

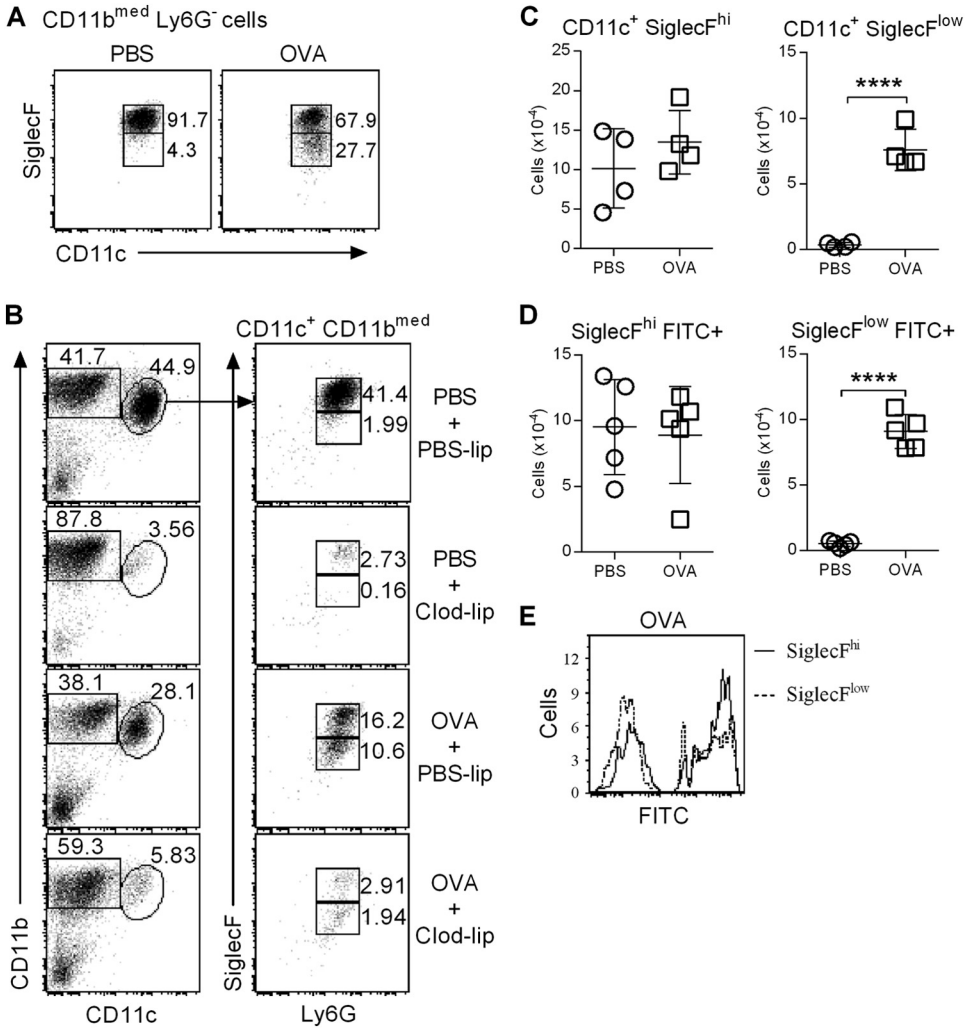

FIG 5 Phagocytic capacity of SiglecF^{low} AMs. (A) Representative flow cytometry histograms demonstrating SiglecF expression on AMs from ALI and non-ALI mice. (B) Representative plots demonstrating depletion of SiglecF^{low} and SiglecF^{hi} AMs in the airway 2 days following i.n. clodronate treatment. BAL fluid cells were collected and stained with fluorescently tagged anti-CD11c, anti-CD11b, anti-Ly6G, and anti-SiglecF MAbs. The data represent results of one of two independent experiments. lip, liposomes. (C) Quantification of total SiglecF^{hi} and SiglecF^{low} AMs in mice with or without ALI. Data represent means ± SD (four mice per group). ****, $P < 0.0001$ (by Student's *t* test). (D) Quantification of FITC$^+$ SiglecF^{hi} and SiglecF^{low} AMs in mice with or without ALI following i.n. administration of FITC-labeled latex beads. Data represent means ± SD of the results of one of two independent experiments (five mice per group). ****, $P < 0.0001$ by Student's *t* test. (E) Representative plot demonstrating FITC fluorescence on SiglecF^{hi} and SiglecF^{low} AMs collected 4 h following i.n. administration of FITC-labeled latex beads in mice with OVA-induced ALI.

somal clodronate, confirming their phagocytic capacity and suggesting that they respond to bacterial infections similarly to traditional SiglecF^{hi} AMs. The critical importance of AM-mediated clearance of pneumococci from the lung has previously been demonstrated by us and others (39, 40). Indeed, increased susceptibility of 129sv mice to pulmonary pneumococcal infections can be attributed to ineffective clearance by AMs (10). SiglecF^{low} AMs have recently been described during the fibrotic phase of bleomycin-induced acute lung injury and were hypothesized to be newly recruited cells that represented an early stage of AM differentiation (19). Likewise, a similar cell population was found in allergic humans following bronchoscopic allergen challenge and were also proposed to be monocyte derived (26). The precise role of this SiglecF^{low} AM population during recovery from inflammatory events in the lung, such as asthma, or during pulmonary infections has yet to be elucidated, though the lower expression of the SiglecF inhibitory receptor (41, 42) supports the hypothesis that these cells may be transitioning to an anti-inflammatory AM phenotype (43, 44). Taking the data together, it is possible that not only increased bacterial clearance but also decreased tissue injury is responsible for the enhanced survival after ALI and bacterial challenge that was observed in our study. Decreased injury could be due to reduced Toll-like receptor signaling (34) and/or heightened production of homeostatic anti-inflammatory cytokines, such as IL-10, TGF-β1, IL-22, and amphiregulin, following ALI. Although no IL-10, IL-22, or amphiregulin was detectable on day 10 following ALI (data not shown), levels of lung TGF-β were significantly increased (Fig. 2B). Further detailed studies, including cell transfer experiments and neutralizing antibody treatments, would be required to further characterize the development of increased resistance in the inflamed lung and the precise role of the SiglecF^{low} AM population that appears following ALI.

The novel finding that ALI enhanced resistance to pulmonary pneumococcal infection was surprising and seemingly opposite what is believed from clinical experience. Although chronic pulmonary diseases in humans, such as COPD and asthma, may increase the risk for developing invasive pneumococcal disease, mortality rates following infection appear to be unaffected in these patients (45). In addition, patients with COPD have been found to be less likely to develop complications such as pleural effusion or multilobar infiltrates following pneumococcal pneumonia (46). In a cohort of patients with community-acquired pneumonia, it was similarly found that patients with asthma presented with less-severe pneumonia, had a reduced length of hospital stays, and had no change in overall mortality compared to patients without asthma (47). Thus, the clinical studies indicate that patients with asthma actually have the same number of complications from pneumococcal infection or fewer complications than nonasthmatic individuals.

In conclusion, we report that allergic inflammation promoted enhanced clearance of pneumococci from the lung after infection and protected mice against lethal pulmonary pneumococcal infection. The enhanced survival was associated with reduced proinflammatory cytokine production and increased TGF-β expression, which, in combination with blunted neutrophil recruitment, led to diminished inflammation in the lung. Increased resistance was also associated with an accumulation of SiglecF^{low} AMs which added to the pool of phagocytes in the airway at the time of infection. Further studies should elucidate the precise mechanism of protection from pneumococcal pneumonia following acute inflammation in the lung.

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