Aeroallergen-induced IL-33 predisposes to respiratory virus–induced asthma by dampening antiviral immunity

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Background: Frequent viral lower respiratory infections in early life are an independent risk factor for asthma onset. This risk and the development of persistent asthma are significantly greater in children who later become sensitized.
Objective: We sought to elucidate the pathogenic processes that underlie the synergistic interplay between allergen exposures and viral infections.
Methods: Mice were inoculated with a murine-specific *Pneumovirus* species (pneumonia virus of mice [PVM]) and exposed to low-dose cockroach extract (CRE) in early and later life, and airway inflammation, remodeling, and hyperreactivity assessed. Mice were treated with anti–IL-33 or apyrase to neutralize or block IL-33 release.
Results: PVM infection or CRE exposure alone did not induce disease, whereas PVM/CRE coexposure acted synergistically to induce the hallmark features of asthma. CRE exposure during viral infection in early life induced a biphasic IL-33 response and impaired IFN-α and IFN-λ production, which in turn increased epithelial viral burden, airway smooth muscle growth, and type 2 inflammation. These features were ameliorated when CRE-induced IL-33 release was blocked or neutralized, whereas substitution of CRE with exogenous IL-33 recapitulated the phenotype observed in PVM/CRE-coexposed mice. Mechanistically, IL-33 downregulated viperin and interferon regulatory factor 7 gene expression and rapidly degraded IL-1 receptor–associated kinase 1 expression in plasmacytoid dendritic cells both *in vivo* and *in vitro*, leading to Toll-like receptor 7 hyporesponsiveness and impaired IFN-α production.
Conclusion: We identified a hitherto unrecognized function of IL-33 as a potent suppressor of innate antiviral immunity and demonstrate that IL-33 contributes significantly to the synergistic interplay between respiratory virus and allergen exposures in the onset and progression of asthma. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Pneumonia virus of mice, IL-33, type 2 innate lymphoid cell, antiviral, respiratory syncytial virus, interferon, plasmacytoid dendritic cell*

Pathologically, asthma is characterized by airway inflammation and airway remodeling; structural changes including goblet cell metaplasia, increased deposition of extracellular matrix proteins, and increased airway smooth muscle (ASM) mass. These features collectively contribute to airway narrowing, loss in lung function, and airway hyperreactivity (AHR) and are poorly responsive to conventional therapies.[1,2] The inflammatory response is typically of a type 2 cytokine profile, which promotes the recruitment and survival of key effector cells, such as eosinophils and mast cells, and can induce features of airway remodeling through direct activation of airway epithelial and mesenchymal cells.[3,4] Both $CD4^+$ T_H2 cells and type 2 innate lymphoid cells (ILCs) produce the type 2 cytokines IL-5 and IL-13[5] in response to the tissue alarmin IL-33 and work collaboratively to affect their teleological role, namely antihelminthic immunity.[6] However, why type 2 immunity is increased during acute exacerbations of asthma, most commonly triggered by a viral respiratory tract infection, remains less clear.

Epidemiologic studies have reproducibly demonstrated that severe/frequent viral lower respiratory tract infections (vLRIs) in early life are a major independent risk factor for asthma inception.[7-9] Notably, this association is markedly increased in children who later become sensitized to food allergens or aeroallergens and, significantly, confers the greatest risk for progression to asthma that is severe, persistent, or both.[10-12] The underlying mechanisms remain poorly defined, although it is likely that the synergistic interaction between allergen exposures and viral respiratory tract infection is underpinned by genetic defects, functional defects, or both in a shared antiviral immune pathway necessary for viral control in early life.[11] Phenotypically, this can manifest as a deficiency in production of the antiviral cytokines IFN-α, IFN-β, and IFN-λ by leukocytes and airway epithelial cells (AECs),[13-15] although whether such defects are virus specific, genetic, or acquired (eg, as a consequence of a type 2 inflammatory milieu) remains highly contentious.[16-18]

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Supported by an equipment grant (Rebecca L. Cooper Medical Research Foundation), an Australian Infectious Disease Research Excellence Award awarded (to S.P., J.P.L, and J.W.U.) and an Australian Research Council Future Fellowship (to S.P.).
Disclosure of potential conflict of interest: A. Haque has received a grant from the National Health and Medical Research Council (1028641). J. W. Upham is on advisory boards for Menarini Pharmaceuticals, Novartis, AstraZeneca, and GlaxoSmithKline. S. Phipps has received a grant from the National Health and Medical Research Council Australia and Pfizer. The rest of the authors declare that they have no relevant conflicts of interest.
Received for publication July 10, 2015; revised January 25, 2016; accepted for publication February 9, 2016.
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0091-6749/$36.00
© 2016 American Academy of Allergy, Asthma & Immunology
http://dx.doi.org/10.1016/j.jaci.2016.02.039

Abbreviations used
AEC: Airway epithelial cell
AHR: Airway hyperreactivity
ASM: Airway smooth muscle
BALF: Bronchoalveolar lavage fluid
CRE: Cockroach extract
HDM: House dust mite
ILC: Innate lymphoid cell
IL1RL1: IL-1 receptor-like 1
IRAK: IL-1 receptor–associated kinase 1
ISG: Interferon-stimulated gene
pDC: Plasmacytoid dendritic cell
pfu: Plaque-forming unit
PVM: Pneumonia virus of mice
RSV: Respiratory syncytial virus
TLR: Toll-like receptor
vLRI: Viral lower respiratory tract infection

Plasmacytoid dendritic cells (pDCs) are among the first cells to respond to a viral infection, sensing viral RNA and producing vast amounts of IFN-α/β downstream of Toll-like receptor (TLR) 7 activation.[19,20] Defects in pDC and TLR7 responsiveness are associated with bronchiolitis, infant wheeze, and childhood asthma.[21,22] In recent studies, we have used the natural mouse pathogen pneumonia virus of mice (PVM), which, unlike human respiratory syncytial virus (RSV), replicates more readily in mice, to induce the more severe symptoms of infantile RSV bronchiolitis.[20,23] *Tlr7* gene deletion delayed the antiviral response, causing severe viral bronchiolitis and release of the alarmin IL-33 in early life, and consequently, an asthma-like pathology developed on viral challenge in later life.[20,24] Of note, the transfer of *Tlr7*-sufficient, but not deficient, pDCs ameliorated bronchiolitis, suggesting that perturbations to the pDC compartment in early life predispose to AEC damage and IL-33 release and hence might underlie the association between severe vLRI and asthma onset.[21] Intriguingly, IL-33 and its receptor, IL-1 receptor-like 1 (IL1RL1, also known as ST2), are susceptibility loci for wheezing in infancy (and asthma),[25,26] although the molecular basis for this association is not known. This led us to question whether allergen-induced release of IL-33 increases the severity of virus-induced bronchiolitis by dampening pDC-orchestrated innate antiviral immunity and to assess whether this early-life interaction causes persistent changes to host immunity that underlie disease progression in response to viral challenge, allergen challenge, or both in later life.

METHODS

Experimental procedures

A detailed description of the materials and procedures used is provided in the Methods section in this article's Online Repository at www.jacionline.org.

Induction of virus- plus allergen-coinduced asthma and associated perturbations

Specific pathogen-free BALB/c mice or 4C13R mice[27] were inoculated with PVM (strain J3666; 1 plaque-forming unit [pfu] in early life and 20 pfu in later life) or cockroach extract (CRE; 1 μg), as previously described[20,28] and as outlined in the study design (Fig 1, *A*). In some experiments mice were exposed to house dust mite (HDM) extract (5 or 100 μg), LPS (186 pg), recombinant IL-33 (10 ng), anti–IL-33/isotype control antibody (200 μg), apyrase (4 U/mL), or pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (100 μm in 10 μL). All studies were approved by the University of Queensland Animal Ethics Committee. Sample processing is described in the Methods section in this article's Online Repository.

Flow cytometry

Lungs were digested to single-cell suspensions, as previously described,[24] and then incubated with anti-FcγRIII/II before incubation with fluorochrome-conjugated antibodies at 4°C for 30 minutes, as described in the Methods section in this article's Online Repository.

Histology and immunohistochemistry

Paraffin-embedded lung sections were prepared, as previously described,[20] and then stained with Chromotrope 2R, periodic acid–Schiff, Masson trichrome, anti–IL-33, anti-PVM G protein, anti–α-smooth muscle actin, or anti-periostin, as described in the Methods section in this article's Online Repository.

Measurement of protein expression

IL-33, IFN-λ2/3, IL-12p40, IFN-γ, IL-13, and IL-5 were quantified by means of ELISA. IFN-α and IL-13 were quantified by using a cytometric bead array. IgG_{1a} was detected by using an in-house ELISA system.[28]

Quantitative real-time PCR

Total RNA was isolated with TRI Reagent solution, followed by phenol-chloroform extraction. Reverse transcription was performed with M-MLV reverse transcriptase and random primers. Quantitative RT-PCR was performed with SYBR Green by using the primers described in Table E1 in this article's Online Repository at www.jacionline.org.

Airway function assessment

Airways resistance was determined by using a forced oscillation technique in response to methacholine, as previously described.[29]

Type 2 ILC culture

Type 2 ILCs were FACS sorted from PVM/CRE-coexposed mice at 10 dpi. Cells were cultured with IL-2 (30 ng/mL) and preincubated with IFN-α (5000 U/mL) before stimulation with IL-33 (30 ng/mL).

pDC culture

Bone marrow–derived pDCs were generated, as described previously.[20] pDCs were preincubated with IL-33 (3 ng/mL) and then stimulated with imiquimod (3 μg/mL).

Statistical analyses

Data were analyzed with a Student *t* test, 1-way ANOVA with a Tukey *post hoc* test, or 2-way ANOVA with a Sidak *post hoc* test, as appropriate, by using GraphPad Prism software (version 5.0; GraphPad Software, La Jolla, Calif). A *P* value of less than .05 was considered statistically significant.

RESULTS

Pneumovirus and allergen coexposure synergize to promote asthma onset and progression

To interrogate the synergistic interaction between a viral respiratory tract infection and allergen exposures, we first identified a dose of CRE that by itself would not induce allergic inflammation. Because we developed a previous mouse model of

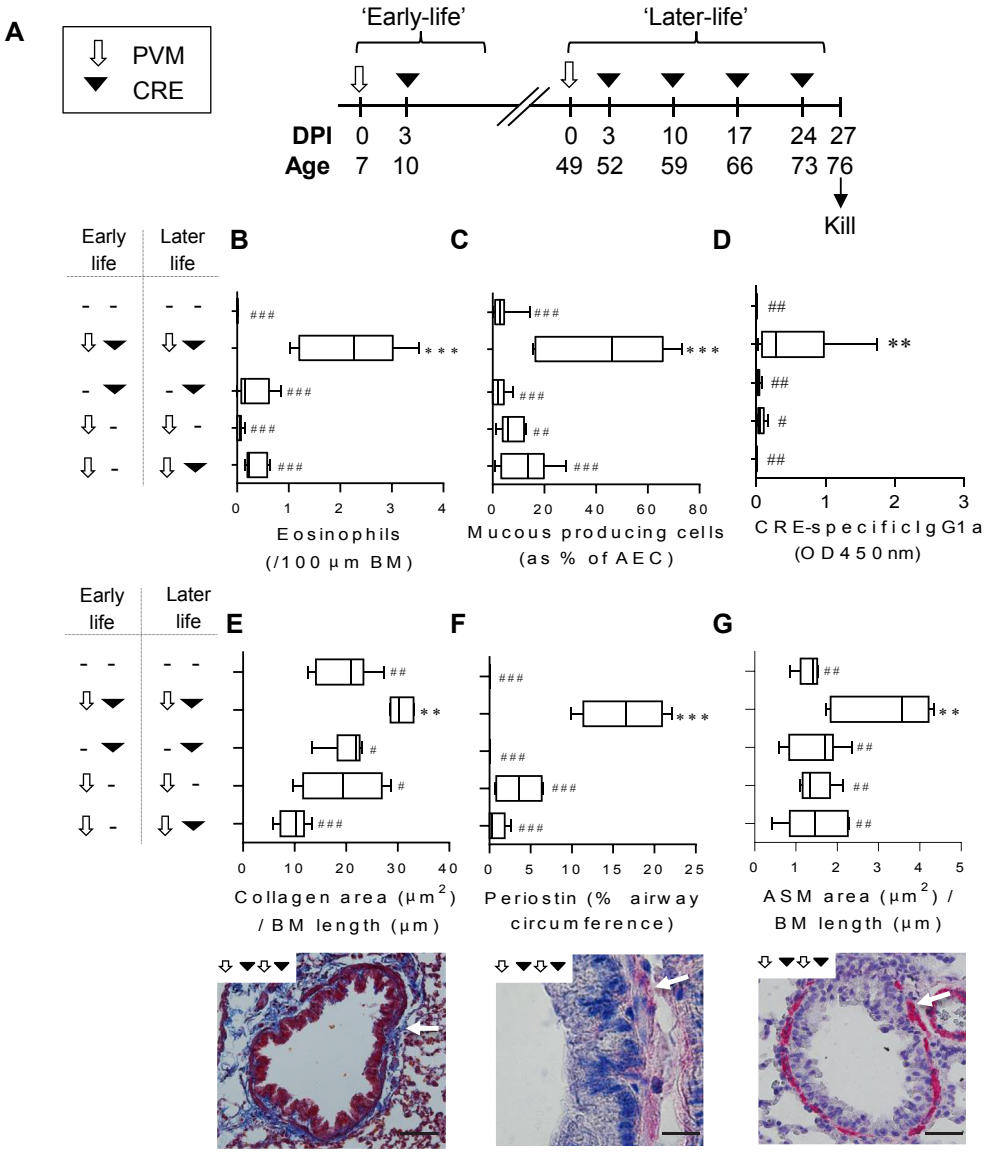


FIG 1. *Pneumovirus* and allergen coexposure synergize in both early and later life to promote type 2 inflammation and airway remodeling. **A,** Study design. **B,** Peribronchial eosinophils expressed per 100 μm of the epithelial basement membrane *(BM)*. **C,** Mucus-secreting cells as a percentage of AECs. **D,** Serum levels of cockroach-specific IgG_{1a} measured by ELISA. **E,** *Top panel*, Peribronchial collagen area; *bottom panel*, representative micrograph (×400 magnification). *Scale bar* = 50 μm. *White arrow* indicates collagen *(blue)*. **F,** *Top panel*, Peribronchial periostin; *bottom panel*, representative micrograph (×1000 magnification). *Scale bar* = 10 μm. *White arrow* indicates periostin *(pink)*. **G,** *Top panel*, Peribronchial ASM area; *bottom panel*, representative micrographs of ASM (×400 magnification). *Scale bar* = 50 μm. *White arrow* indicates ASM *(pink)*. *BM*, Basement membrane. Data are means ± SEMs and representative of 3 independent experiments (n = 6-9 mice per group). **P* < .05, ***P* < .01, and ****P* < .001 compared with vehicle-treated mice. #*P* < .05, ##*P* < .01, and ###*P* < .001 compared with PVM/CRE/PVM/CRE mice.

virus-induced asthma by inoculating mice with PVM at 7 and 49 days of age,[24] we elected to expose mice to CRE (intranasal route) at 10 days of age (ie, 3 days after the primary virus inoculation; early life), followed by a series of CRE exposures (challenges in later life) at 52, 59, 66, and 73 days of age (ie, starting 3 days after virus challenge; see study design in Fig 1, *A*). Repeated exposure to 10 or 100 μg but not 0.1 or 1 μg of CRE (in the absence of PVM infection) induced peribronchial eosinophilia and mucus hypersecretion (see Fig E1 in this article's Online Repository at www.jacionline.org). Therefore we elected to use 1 μg of CRE for all further studies. To assess for a synergistic effect between PVM and CRE, we superimposed a low-dose PVM infection, which by itself did not induce features of type 2 inflammation (Fig 1, *B-D*, and see Fig E2 in this article's Online Repository at www.jacionline.org) onto the low-dose CRE exposure model (Fig 1, *A*). Eosinophilic inflammation, mucus hypersecretion, CRE-specific IgG_{1a} levels (Fig 1, *B-D*), and mucosal mast cell numbers (data not shown) were greatest in mice exposed to both PVM and CRE. By contrast, these features of type 2 inflammation were absent in mice exposed to PVM

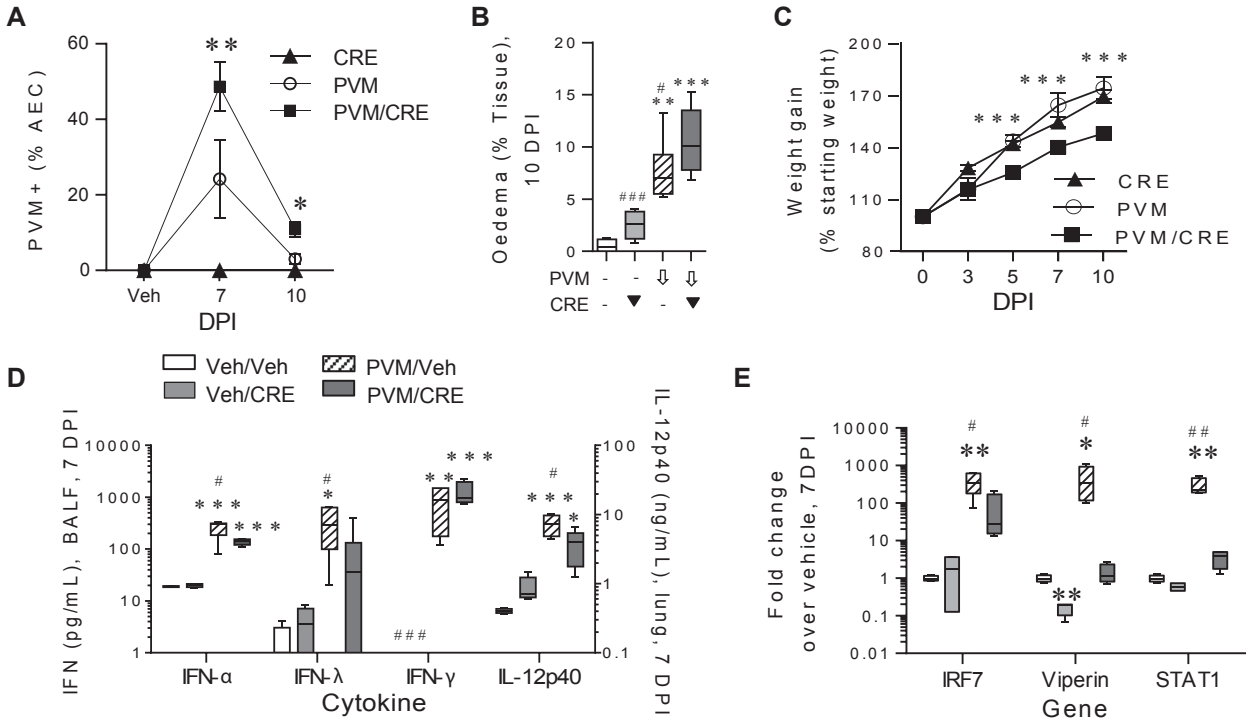


FIG 2. Early-life CRE exposure increases viral load and dampens antiviral cytokine production. **A,** Viral load in AECs detected by using immunohistochemistry and enumerated as a percentage of total AECs. **B,** Edema in lung parenchyma. **C,** Weight gain. **D,** IFN-α, IFN-λ, and IFN-γ levels in BALF and IL-12p40 levels in lung homogenate. **E,** mRNA expression of interferon-regulatory factor 7 *(IRF7)*, viperin, and signal transducer and activator of transcription 1 *(STAT1)* in lungs relative to those in vehicle-treated mice. Data are means ± SEMs and representative of 2 to 3 experiments (n = 4-6 mice per group). **P* < .05, ***P* < .01, and ****P* < .001 compared with vehicle-treated mice. #*P* < .05, ##*P* < .01, and ###*P* < .001 compared with PVM/CRE mice.

alone or low-dose CRE alone in both early and later life or low-dose CRE alone in later life (Fig 1, *B-D*, and see Fig E2). Similarly, features of airway remodeling, including collagen and periostin deposition, and ASM remodeling (Fig 1, *E-G*) were only evident in PVM/CRE-coexposed mice.

To compare our findings to contemporary acute models of allergen-induced disease,[30] we exposed a group of mice to high-dose (100 μg) CRE in later life alone. Strikingly, this regimen elicited type 2 inflammation but was not sufficient to elicit features of airway remodeling (see Fig E2). Thus low-dose virus and low-dose allergen coexposures acted synergistically to induce all of the cardinal features of asthma.

Virus and allergen exposure in early and later life are necessary for maximal asthma severity

We next addressed the relative contribution of the virus and allergen exposures in early and later life. PVM/CRE coexposure in early life alone was not sufficient to induce type 2 inflammation or airway remodeling compared with the vehicle-treated group (see Fig E2). The importance of both the virus and allergen challenge for asthma progression was confirmed when the omission of either exposure significantly diminished airway inflammation, airway remodeling, and AHR when assessed at 76 days of age (see Fig E2). These findings led us to question the importance of the CRE exposure during primary viral infection. Remarkably, when this exposure was omitted, type 2 inflammation, airway remodeling, and AHR were completely absent (Fig 1, final row), even though the mice had been infected with PVM in early and later life and then challenged repeatedly with CRE. Taken together, these data suggested that the CRE exposure in early life fundamentally altered the nature of the host response to secondary virus and/or allergen exposure in later life and that this effect was necessary for disease progression.

Allergen exposure increases viral load and lung tissue damage and dampens antiviral cytokine production

Aeroallergens are known to rapidly induce innate inflammation in the airways.[28,31,32] We hypothesized that allergen-induced inflammation might perturb the host's antiviral response to PVM, thus increasing the severity of viral bronchiolitis. Viral load in the airway epithelium was significantly greater and persisted for longer in PVM/CRE-coexposed mice compared with that seen in mice inoculated with virus alone (Fig 2, *A*, and see Fig E3, *A*, in this article's Online Repository at www.jacionline.org). In children with severe RSV bronchiolitis, the virus can spread to the parenchyma[33]; however, few PVM-immunoreactive cells were observed in the alveoli (data not shown). Strikingly, PVM/CRE coexposure stunted weight gain and heightened tissue edema, indicative of severe disease (Fig 2, *B* and *C*). The increased viral illness in PVM/CRE-coexposed mice was associated with significantly lower IFN-α, IFN-λ, and IL-12p40, but not IFN-γ, production in bronchoalveolar lavage fluid (BALF; Fig 2, *D*) and attenuated transcription of interferon-stimulated genes (ISGs) involved in TLR7 and

type I interferon receptor signaling, including *Irf7*, *Viperin*, and *Stat1* (Fig 2, *E*). Intriguingly, CRE exposure alone significantly downregulated the expression of *Viperin*, a component of the TLR7 signaling cascade in pDCs.[34] Although TLR7-mediated activation of pDCs is critical for host defense against acute PVM infection,[20] CRE exposure did not affect pDC numbers in the lung (see Fig E3, *B*).

Type 2 inflammation and ASM remodeling are increased in PVM/CRE-coexposed mice in early life

Allergen exposure is well known to induce the release of IL-33 to promote type 2 inflammation.[35] Analyzing IL-33 protein expression across the time course of infection revealed a significant increase in lung homogenates at 3 dpi (sampling 2 hours after CRE exposure) and 7 dpi in the coexposed mice but not in those exposed to PVM alone (Fig 3, *A*, upper panel). Although CRE exposure did not increase lung IL-33 expression at 3 dpi, it did increase airway luminal IL-33 levels measured in the BALF at this time, both in PVM-infected mice and noninfected mice (Fig 3, *B*). Consistent with other reports,[36] under homeostatic conditions, IL-33 was expressed predominantly in resident myeloid cell populations and alveolar (but not airway) epithelial cells (Fig 3, *C*, top left panel). However, by 7 dpi, IL-33–immunoreactive AECs were evident in PVM alone and PVM/CRE-exposed mice (Fig 3, *C*, and see Fig E3, *C*), which was notable as a second phase of IL-33 release occurred at 10 dpi (Fig 3, *B*). In contrast to IL-33, expression of IL-25 and thymic stromal lymphopoietin was unaffected by PVM exposure, CRE exposure, or both (data not shown). Critically, the IL-33 response was associated with increased numbers of type 2 ILCs and eosinophils ($CD4^+$ and $CD8^+$ T-cell numbers were unaffected, data not shown), and type 2 cytokine expression (Fig 3, *D-F*, and see Fig E4, *A*, in this article's Online Repository at www.jacionline.org). Using IL-4/IL-13 reporter mice,[27] we revealed that $CD8^+$, but not $CD4^+$, T cells contributed to IL-4 and IL-13 production. However, the majority of type 2 ILCs were IL-13^+, and the proportion of IL-13–expressing type 2 ILCs, but not $CD4^+$ or $CD8^+$ T cells, increased in PVM/CRE-coexposed mice (see Fig E4, *B*). Similar to our observations of ASM remodeling in later life (Fig 1, *G*), PVM infection or CRE exposure alone had no effect on ASM growth, whereas coexposure induced a 3-fold increase in ASM area (Fig 3, *G*).

Increased viral load is not sufficient to induce type 2 inflammation and ASM remodeling

To address whether increased viral load was causally related to the development of ASM growth, we first inoculated mice with a 10× higher dose of PVM. Although this increased the viral load and induced severe illness and mortality (Fig 4, *A* and *B*), it was not sufficient to increase IL-33 release at 3 dpi (even when the dose was increased 100-fold [data not shown]) or induce ASM growth (Fig 4, *C* and *D*). We then assessed whether HDM was able to affect viral load and ASM growth at 7 and 10 dpi, respectively (when these pathologies peaked). Substitution of CRE with HDM significantly increased viral load similar to CRE coexposure but had no effect on ASM growth (Fig 4, *E* and *F*), which was consistent with a lack of effect on antiviral cytokine expression, type 2 ILCs in the lungs, or IL-33 release (Fig 4, *G-I*). Notably, HDM induced IL-33 release was detectable at 7 dpi, which is consistent with the findings of others (data not shown).[28,31,37-39] Because the level of endotoxin in the CRE was high (395 EU per 100 μg of protein), we coexposed mice to PVM and an equivalent dose of LPS. LPS induced a massive IFN-α response and decreased viral load (Fig 4, *H* and *I*), suggesting LPS contamination of CRE was not the cause of impaired antiviral immunity. Taken together, our findings suggested that ASM growth and the onset of type 2 inflammation was associated with an IFN-α/λ^{low}IL-33^{high} cytokine microenvironment rather than viral load.

Anti–IL-33 prevents type 2 inflammation and remodeling in response to virus and allergen coexposure in early and later life

Mice were treated with a neutralizing antibody in both early and later life to assess the contribution of IL-33 (see Fig E5, *A*, in this article's Online Repository at www.jacionline.org). Although mice treated with an isotype-matched control had all of the hallmark features of asthma, treatment with anti–IL-33 abolished these features (see Fig E5, *B-E*). Similarly, anti–IL-33 treatment in early life ablated ASM growth and the onset of type 2 inflammation (Fig 5 and see Fig E5, *F*). As expected, anti–IL-33 had no effect in mice infected with PVM alone.

IL-33 is a negative regulator of innate antiviral immunity

We hypothesized that CRE might dampen innate antiviral immunity through IL-33 release. Strikingly, anti–IL-33 reversed the increased viral burden, dampened IFN-α and IFN-λ production, and attenuated ISG expression caused by CRE exposure but did not alter pDC or CD8 T-cell numbers in the lung (Fig 6, *A-C*, and see Fig E5, *G* and *H*). In contrast, anti–IL-33 had no effect on the antiviral response in mice infected with PVM alone. CRE-induced IL-33 release occurs downstream of ATP-mediated purinergic receptor activation.[35] In our model treatment with apyrase (to catalyze the hydrolysis of ATP) significantly decreased IL-33 levels in BALF (2 hours after CRE exposure; see Fig E6, *A*, in this article's Online Repository at www.jacionline.org) and significantly decreased viral load at 7 dpi in the airway epithelium of PVM/CRE-coexposed mice (Fig 6, *D*). Treatment with the broad-spectrum P2R antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid had the same effect (see Fig E6, *A* and *B*), further implicating nucleoside/purinergic receptor signaling in mediating IL-33 release. As with anti–IL-33, blocking IL-33 release recapitulated IFN-α production and ISG expression in the lung (Fig 6, *E* and *F*), decreased IL-13 expression (data not shown), and ablated ASM growth (Fig 6, *G*), again highlighting the protective nature of an IFN-α/λ^{high}IL-33^{low} cytokine environment. This led us to question whether IFN-α suppresses IL-33–induced cytokine production by type 2 ILCs. Notably, FACS-purified type 2 ILCs expressed the type I interferon receptor (see Fig E4, *A*) and produced less IL-5 and IL-13 in response to IL-2/IL-33 stimulation when pretreated with IFN-α (Fig 6, *H*). This response was unrelated to cell death (see Fig E6, *C* and *D*); however, we observed that IFN-α attenuated IL-2/IL-33–induced upregulation of ST2 (Fig 6, *I*).

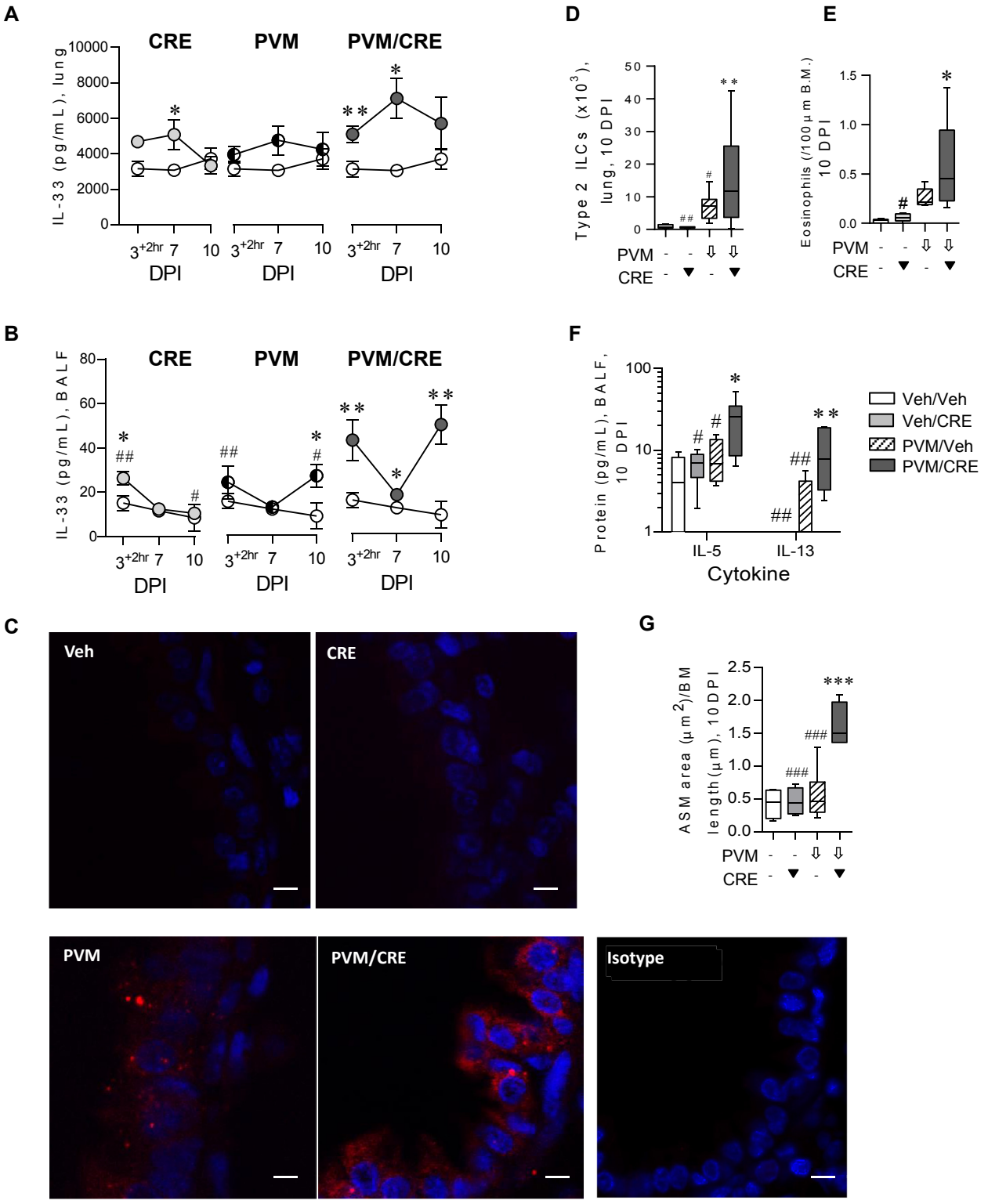


FIG 3. Type 2 inflammation in early life is increased in PVM/CRE-coexposed mice. **A** and **B,** IL-33 in lung homogenate (Fig 3, *A*) and BALF (Fig 3, *B*). **C,** Representative micrographs of IL-33 immunostaining plus 4′,6-diamidino-2-phenylindole *(DAPI)* counterstaining. *Scale bar* = 50 μm. **D,** Type 2 ILCs in lung (Lineage$^-$CD45$^+$CD90.2$^+$CD25$^+$ST2$^+$). **E,** Peribronchial eosinophils. **F,** IL-5 and IL-13 in BALF. **G,** ASM area. *BM*, Basement membrane. Data are means ± SEMs and representative of 2 independent experiments (n = 6-8 mice per group). **P* < .05, ***P* < .01, and ****P* < .001 compared with vehicle-treated mice. #*P* < .05, ##*P* < .01, and ###*P* < .001 compared with PVM/CRE mice.

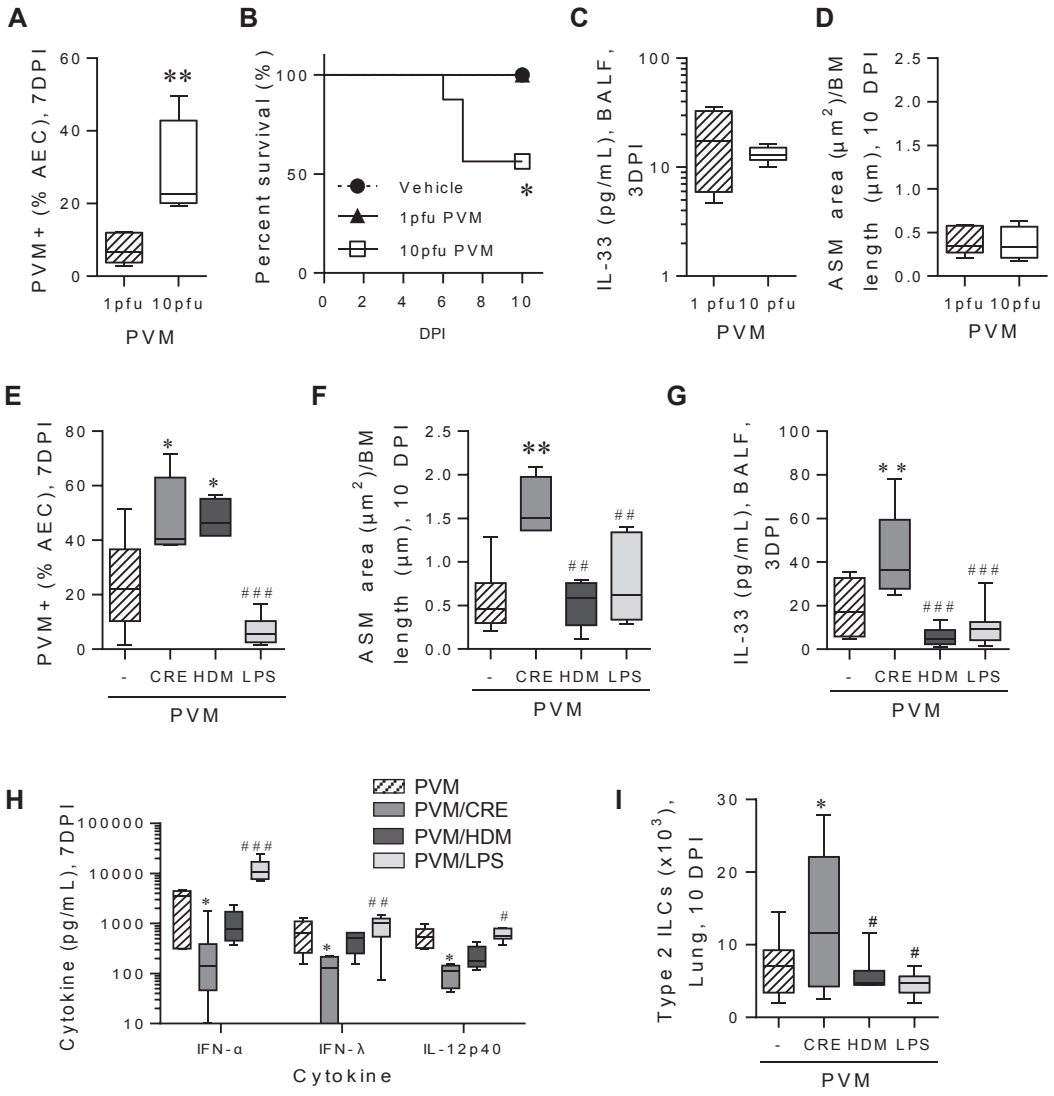


FIG 4. High viral load alone does not promote type 2 inflammation and airway remodeling. **A** and **E,** Viral load in AECs. **B,** Survival curve after intranasal infection with 1 or 10 pfu of PVM. **C** and **G,** IL-33 in BALF. **D** and **F**, ASM area. **H,** IFN-α, IFN-λ, and IFN-γ levels in BALF and IL-12p40 levels in lung homogenate. **I,** Type 2 ILCs in lung. *BM*, Basement membrane. Data are means ± SEMs and representative of 2 independent experiments (n = 6-8 mice per group). **P* < .05, ***P* < .01, and ****P* < .001 compared with PVM-treated mice. #*P* < .05, ##*P* < .01, and ###*P* < .001 compared with PVM/CRE mice.

Exogenous IL-33 increases viral load and decreases IL-1 receptor–associated kinase 1 expression and antiviral cytokine production by pDCs

We next examined the effect of low-dose exogenous IL-33 during acute PVM infection. Similar to CRE, exposure to exogenous IL-33 (10 ng at 3, 4, and 5 dpi; intranasal route) during acute PVM infection did not affect the infiltration of pDCs (see Fig E7, *A* and *B*, in this article's Online Repository at www.jacionline.org); however, it significantly diminished IFN-α and IFN-λ levels in the BALF, increased epithelial viral load, and ablated ISG expression in the lung (Fig 7, *A-D*). Moreover, IL-33/PVM coexposure significantly increased ASM growth in contrast to IL-33 or PVM alone (Fig 7, *E*). Lastly, we questioned whether IL-33 affects TLR7-mediated activation of pDCs by rapidly depleting the intracellular adaptor molecules IL-1 receptor–associated kinase 1 (IRAK1) and viperin.[40,41] Consistent with this possibility, ST2 was expressed on pDCs in bone marrow, lung, and mediastinal lymph nodes (Fig 7, *F*). Notably, in PVM/CRE-coexposed mice, intracellular IRAK1 and viperin expression in pDCs was significantly diminished within 2 hours of CRE administration (Fig 7, *G*). To directly assess the role of IL-33, we stimulated pDCs *in vitro* with IL-33. As shown *in vivo*, IRAK1 expression by pDCs was rapidly decreased (Fig 7, *H*). Additionally, IL-33 treatment significantly diminished the production of IFN-α (Fig 7, *I*) and IL-12p40 (data not shown) in response to TLR7 stimulation. Collectively, these data suggest that IL-33 negatively regulates the early antiviral response by inducing a state of TLR7 hyporesponsiveness in pDCs.

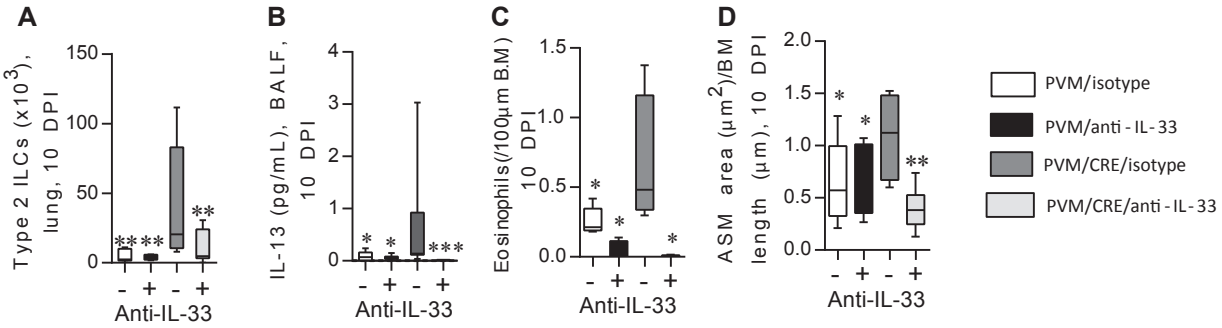


FIG 5. Anti–IL-33 prevents type 2 inflammation and remodeling in response to PVM and CRE coexposure in early life. **A,** Type 2 ILCs in lung. **B,** IL-13 in BALF. **C,** Peribronchial eosinophils. **D,** ASM area. Data are means ± SEMs and representative of 2 experiments (n = 6-7 mice per group). *BM,* Basement membrane. **P* < .05, ***P* < .01, and ****P* < .001 compared with isotype-treated PVM/CRE-coexposed mice.

DISCUSSION

IL-33 and its receptor, ST2, have been reproducibly identified in genetic studies as asthma susceptibility loci, and clinical and experimental studies have revealed a preeminent role for IL-33 in the development and expansion of T_H2 immunity through activation of $CD4^+$ T_H2 cells, type 2 ILCs, and various type 2 effector cells. In the present study we extend this paradigm to show that IL-33 dampens antiviral cytokine production, thus removing an inhibitory tonic and establishing a cytokine microenvironment that is conducive for the expansion of type 2 inflammation and ASM growth. Critically, using a novel preclinical model of asthma, we demonstrate that this dual activity of IL-33 underlies the synergistic effect of respiratory virus and allergen exposures on the development and progression of asthma.

We repeatedly inoculated mice with low-dose virus and allergen to better simulate the natural course of events and demonstrated that each of these environmental exposures in both early and later life was necessary to maximally induce all of the cardinal features of asthma. Several investigators have developed experimental mouse models of virus/allergen coexposure to show that a viral respiratory tract infection facilitates sensitization to an otherwise innocuous allergen; however, in these models, as in ours, allergic sensitization was not apparent until after allergen challenge. We note that analysis from one birth cohort study found that allergic sensitization precedes rhinovirus-induced wheezing.[42] However, it is important to note that although rhinovirus-associated illness occurs in older children, severe RSV bronchiolitis often occurs in the first year of life, when aeroallergen-specific IgE titers remain low.[10] Because an antibody-mediated response did not account for the predisposing effect of CRE in early life, we questioned whether the primary allergen exposure altered the course of the viral respiratory tract infection. Unexpectedly, we found that CRE exposure attenuated antiviral cytokine production and doubled the viral load in the airway epithelium. Treatment of PVM/CRE-coexposed mice with anti–IL-33 or apyrase restored IFN-α production and decreased the viral burden to levels observed with PVM alone, whereas substitution of CRE with exogenous IL-33 attenuated IFN-α production and increased viral load, implicating IL-33 as a potent suppressor of antiviral immunity. Mechanistically, we found that IRAK1 and viperin expression by pDCs was lower *in vivo* in PVM/CRE-coexposed mice and IRAK1 expression was lower *in vitro* after IL-33 treatment. Significantly, IL-33 impaired IFN-α production by pDCs, which is consistent with our earlier report that TLR7 hyporesponsiveness is a feature of asthma.[22] Additionally, CRE or IL-33 exposure decreased *Viperin* and *Irf7* gene expression *in vivo*, whereas anti–IL-33 or apyrase treatment attenuated this downregulation. Because viperin interacts with IRAK1 to induce the nuclear translocation of IRF7 and downstream interferon production by pDCs,[34] our findings illustrate that IL-33 negatively regulates TLR7 signaling by at least 3 key points: viperin, IRAK1, and IRF7.

Presently, the role of type I and III interferons in asthma pathogenesis is highly contentious. The first reports of impaired IFN-β and IFN-λ production by *ex vivo* virus-stimulated AECs from asthmatic patients[13,14] have not been universally replicated by other investigators,[16-18] leading to suggestions that the phenotype is only present in a subpopulation of patients or might stem from the cytokine milieu.[43] In light of our findings, these concepts might not be mutually exclusive because the IL-33 receptor IL1RL1, a common susceptibility locus for asthma,[25] is expressed on AECs[44] and rhinovirus-infected AECs secrete IL-33.[45] Accordingly, it will be important to test whether the increased viral load in nasal lavage fluid, as reported by Jackson et al,[45] is related to the suppressive effects of IL-33 on antiviral immunity.

The phenotype of our early-life model shows a number of striking similarities to pediatric patients with severe therapy-resistant asthma, including impaired type I and III interferon production, increased collagen deposition, and eosinophilic inflammation.[46-48] It remains to be established whether the cause of disease in this patient group relates to severe/frequent vLRIs; however, it is noteworthy that they present with increased IL-33 expression[47] and therefore have an IFN-α/λ^{low}IL-33^{high} cytokine microenvironment, as observed in our model. We and others have shown that type I and III interferons suppress $CD4^+$ T_H2 cytokine production,[49] and here we show that IFN-α suppressed IL-33–induced type 2 cytokine production by type 2 ILCs, in part by preventing ST2 upregulation. Our findings highlight an important counterregulatory process whereby IL-33 suppresses antiviral cytokine production. Such systems are critically dependent on the timing of expression, emphasizing the need to tightly regulate the release of prestored IL-33. Evidently, if IL-33 release is triggered inadvertently, as we show here in response to CRE exposure, this can have profound short- and long-term effects, which

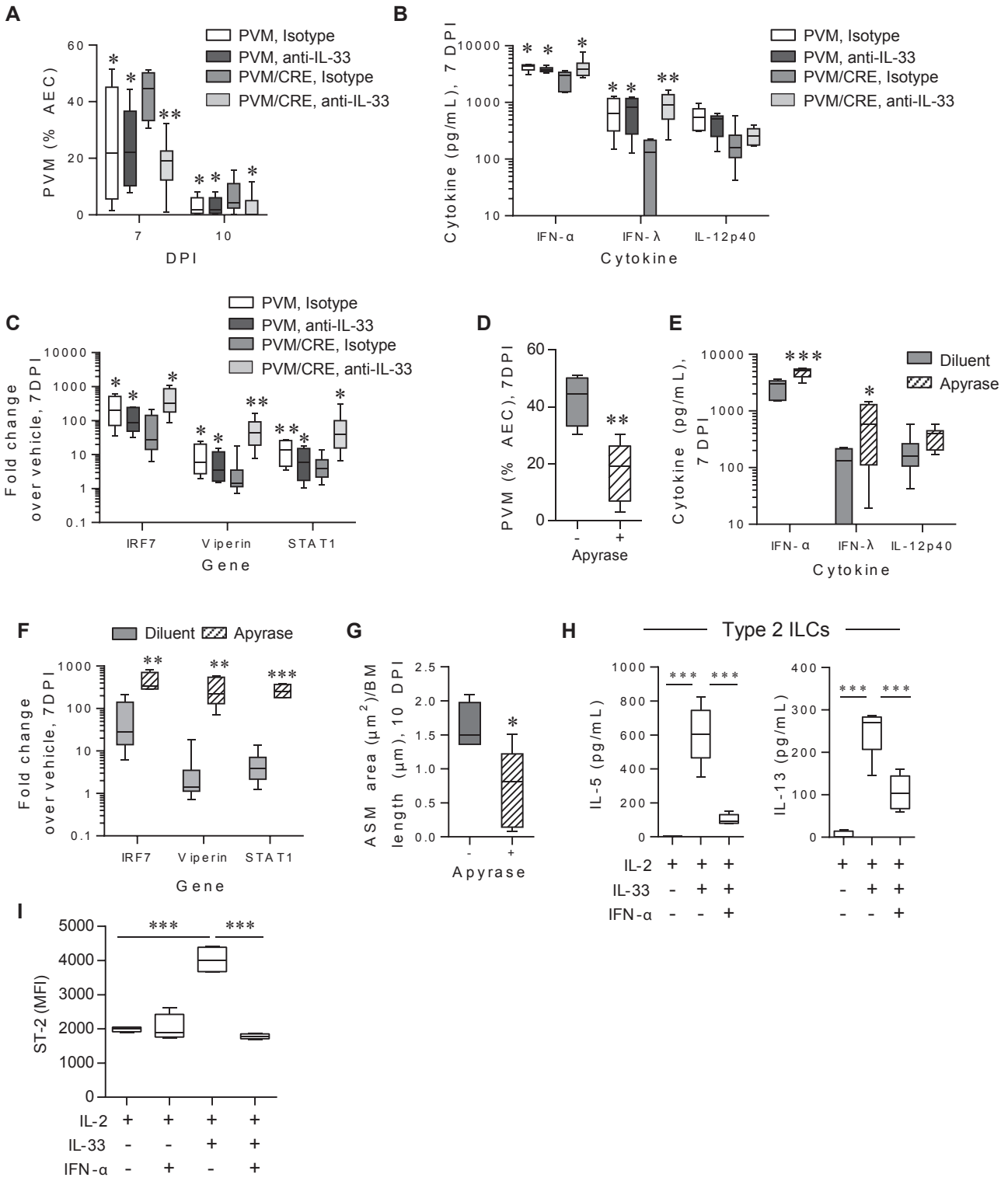


FIG 6. IL-33 blockade in PVM/CRE-coexposed mice prevents excessive viral load and reverses dampened antiviral immunity induced by CRE. **A** and **D,** AEC viral load. **B** and **E,** IFN-α and IFN-λ levels in BALF and IL-12p40 levels in lung homogenate. **C** and **F,** mRNA expression of ISGs relative to vehicle-treated mice. **G,** ASM area. **H,** Type 2 ILCs were treated with IL-2 with or without IFN-α for 30 minutes, followed by culture with IL-33 and IL-2 for 72 hours, before the supernatant was probed for IL-5 and IL-13. **I,** ST2 expression on cultured type 2 ILCs. *MFI,* Mean fluorescence intensity. Data are means ± SEMs and representative of 2 to 3 independent experiments (n = 6-10 mice per group). *BM,* Basement membrane. **P* < .05, ***P* < .01, and ****P* < .001 compared with PVM/CRE/isotype mice or as indicated.

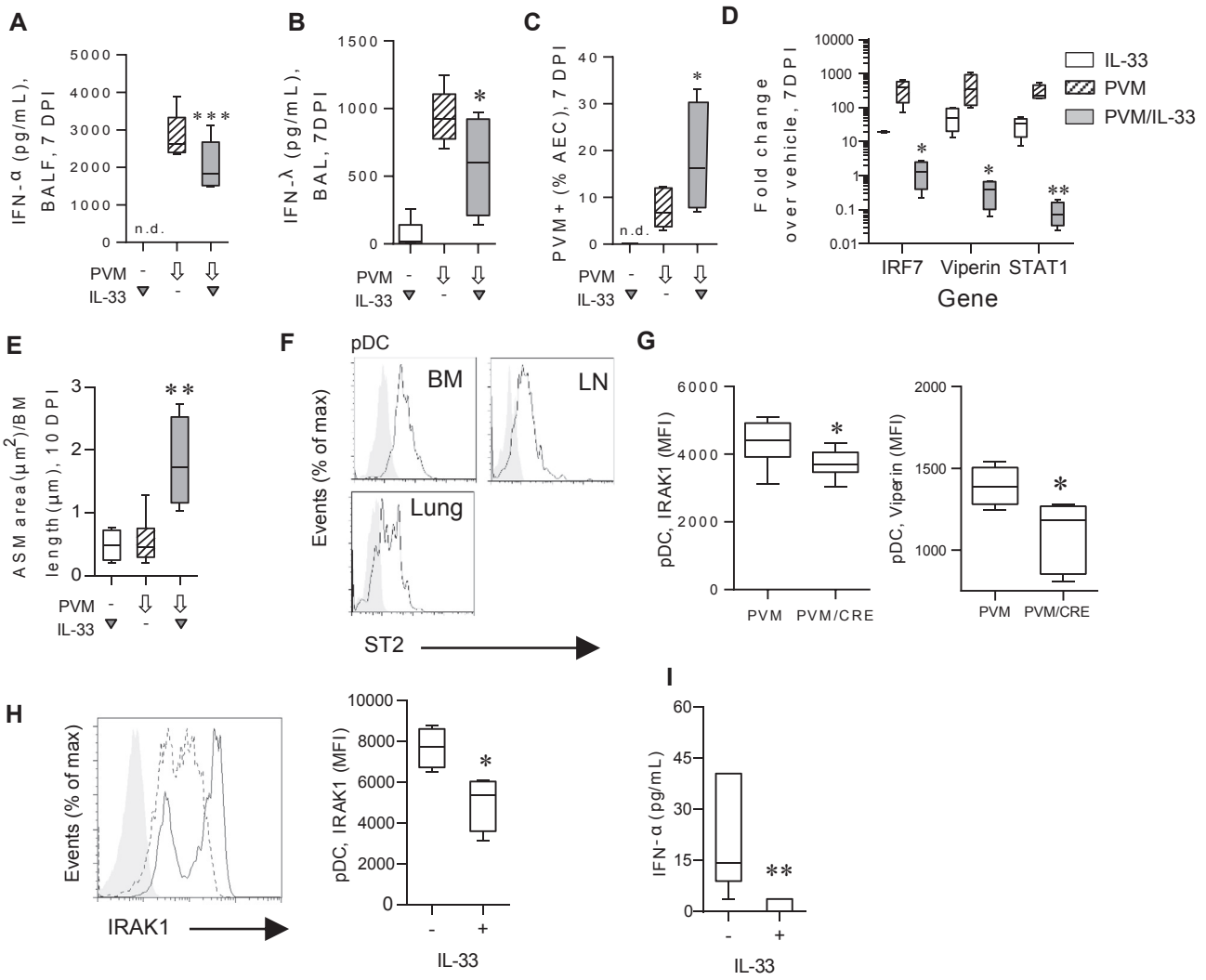


FIG 7. Exogenous IL-33 dampens IFN-α production and increases viral load by decreasing IRAK1 expression and antiviral cytokine production by pDCs. **A** and **B,** IFN-α (Fig 7, *A*) and IFN-λ (Fig 7, *B*) in BALF. **C,** Viral load in AECs. **D,** mRNA expression relative to vehicle-treated mice. **E,** ASM area. **F,** ST2 staining of bone marrow (BM), lung, and mediastinal lymph node (LN) pDCs *(solid line).* Fluorescence minus one for ST2 staining is shown in *gray*. **G,** IRAK1 and viperin intracellular expression in pDC *in vivo* at 2 hours after CRE administration. *MFI,* Mean fluorescence intensity. **H,** Intracellular IRAK1 staining of bone marrow–derived pDCs preincubated with vehicle *(solid line)* or IL-33 (3 ng/mL, *dotted line*) for 0.5 hours. Fluorescence minus one for IRAK staining is shown in *gray*. **I,** IFN-α production in bone marrow–derived pDC cell-culture supernatants. *BM,* Basement membrane. Data are means ± SEMs and representative of 2 independent experiments (n = 7 mice per group). *n.d.,* Not detected. **P* < .05, ***P* < .01, and ****P* < .001 compared with mice exposed to PVM alone or vehicle-treated bone marrow–derived pDCs.

can be exacerbated in subjects with a gain-of-function single nucleotide polymorphism in the IL-33 or IL1RL1 gene.[50] The importance of timing was further emphasized when we switched CRE with HDM; although HDM did induce IL-33, as others have shown, the response was too slow to affect the antiviral response. Thus we predict that exposure to HDM at the time of inoculation would predispose to viral bronchiolitis and subsequent asthma.

In the naive mouse lung IL-33 is primarily expressed by alveolar epithelial cells; however, in response to ovalbumin challenge, several different types of hematopoietic cells also produce IL-33.[36] In our model PVM/CRE coexposure of neonatal mice induced IL-33 release in a biphasic manner, peaking at 3 dpi (ie, 2 hours after CRE exposure) and then at 10 dpi. Notably, CRE alone induced IL-33 release at 3 dpi but not 10 dpi, PVM infection alone induced IL-33 release at 10 dpi only, while in coexposed mice, expression was significantly increased at both time points. PVM infection increased IL-33 expression in AECs, although not until 7 dpi, implicating infiltrating inflammatory cells or alveolar epithelial cells as the source of IL-33 at 3 dpi. This release of IL-33 was ATP dependent[35]; however, the late release, which was associated with PVM infection, might involve a separate mechanism. Indeed, recent reports have shown that the release of active IL-33 can also occur downstream of necroptosis, a programmed form of necrotic cell death, that can be initiated by viral activation of TLR3,[51] which is consistent with the apparent association between late IL-33 release and viral load in our model at 10 dpi.

A striking feature of our model was the development of ASM growth, which to our knowledge has not been observed previously in an experimental model of virus-induced asthma. Furthermore, we demonstrated that this hallmark pathology of asthma began to develop in early life, which is consistent with a recent clinical report in which ASM changes were evident in preschool-aged children with wheeze who are later given a diagnosis of asthma.[52] In fact, evidence of ASM proliferation is infrequent in patients with established asthma,[53] emphasizing the need for new therapies to target prevention in early life, rather than resolution of ASM mass in later life. Neutralization or blockade of IL-33 release significantly decreased the development of ASM remodeling, together with other features of asthma, in both early and later life. IL-33 has been shown to directly induce collagen synthesis from fibroblasts[47]; however, ASM cells do not express ST2,[44] and hence it is likely that the effects of IL-33 on ASM growth in our model were indirect, perhaps being mediated through activation of type 2 ILCs, eosinophils, or both.

In summary, we have developed a novel model to study the molecular processes that underlie the synergistic relationship between vLRI and allergen exposure and the onset of asthma. CRE exposure rapidly induces the release of IL-33, which downregulates components of the TLR7 signaling pathway causing TLR7 hyporesponsiveness in pDCs. The ensuing IFN-α/λ^{low}IL-33^{high} cytokine microenvironment allows for expansion of type 2 inflammation and increased ASM growth in early life. This in turn leads to persistent alterations to resident airway cells, immune cells, or both, which are necessary for disease progression after challenge with virus and allergen in later life. Thus emerging therapies aimed at targeting IL-33 will not only decrease T_H2 inflammation but also likely boost innate antiviral immunity.

We thank Drs Nocka, Kasaian, and Bloom (Pfizer) for supplying anti–IL-33. We also thank Dr Ashik Ullah (Queensland Institute of Medical Research) and Dr Ben Roediger (Centenary Institute) for technical assistance.

Key messages

- **Aeroallergen-induced IL-33 release suppresses antiviral immunity, increasing the severity of viral bronchiolitis.**
- **IL-33 impairs antiviral immunity by degrading IRAK1 and viperin in pDCs, leading to a state of TLR7 hyporesponsiveness.**
- **Coexposure to virus and allergen in early life predisposes to asthma progression in later life.**

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