Enteric helminth-induced type I interferon signaling protects against pulmonary virus infection through interaction with the microbiota

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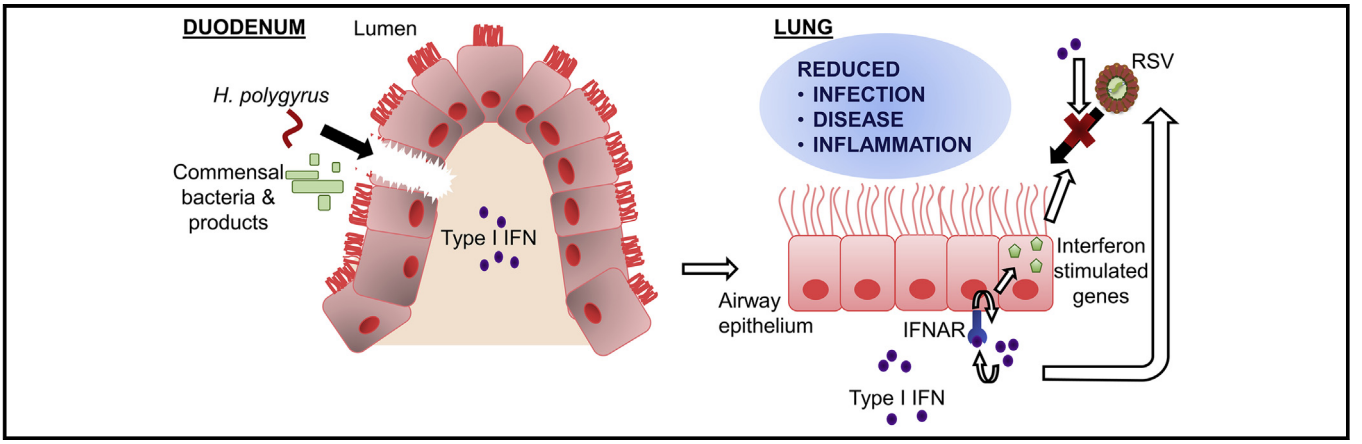
GRAPHICAL ABSTRACT



Background: Helminth parasites have been reported to have beneficial immunomodulatory effects in patients with allergic and autoimmune conditions and detrimental consequences in patients with tuberculosis and some viral infections. Their role in coinfection with respiratory viruses is not clear.

Objective: Here we investigated the effects of strictly enteric helminth infection with *Heligmosomoides polygyrus* on respiratory syncytial virus (RSV) infection in a mouse model.

Methods: A murine helminth/RSV coinfection model was developed. Mice were infected by means of oral gavage with 200 stage 3 *H polygyrus* larvae. Ten days later, mice were infected intranasally with either RSV or UV-inactivated RSV.

Results: *H polygyrus*–infected mice showed significantly less disease and pulmonary inflammation after RSV infection associated with reduced viral load. Adaptive immune responses, including T_H2 responses, were not essential because protection against RSV was maintained in *Rag1*$^{-/-}$ and *Il4rα*$^{-/-}$ mice. Importantly, *H polygyrus* infection upregulated expression of type I interferons and interferon-stimulated genes in both the duodenum and lung, and its protective effects were lost in both *Ifnar1*$^{-/-}$ and germ-free mice, revealing essential roles for type I interferon signaling and microbiota in *H polygyrus*–induced protection against RSV.

Conclusion: These data demonstrate that a strictly enteric helminth infection can have remote protective antiviral effects in the lung through induction of a microbiota-dependent type I interferon response. (J Allergy Clin Immunol 2017;140:1068-78.)

Key words: *Respiratory syncytial virus, helminths,* Heligmosomoides polygyrus*, type I interferon, microbiome*

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Abbreviations used
cDC: Conventional dendritic cell
HES: *Heligmosomoides polygyrus* excretory-secretory
IL-4R: IL-4 receptor
ISG: Interferon-stimulated gene
mCRAMP: Murine cathelicidin-related antimicrobial peptide
NK: Natural killer
OAS: 2′-5′-Oligoadenylate synthetase
RAG: Recombination-activating gene
RSV: Respiratory syncytial virus
SPF: Specific pathogen-free
UV-RSV: UV-inactivated RSV

Respiratory syncytial virus (RSV) is a major respiratory pathogen. It infects nearly all infants by the age of 2 years[1] but does not induce lasting immunity and leads to recurrent infections throughout life. Worldwide, it is estimated that 33.4 million children under 5 years of age experience RSV-induced lower respiratory tract infection (LRTI) annually, and 10% of these require hospitalization, resulting in up to 199,000 deaths.[2-4] There is also major morbidity and mortality caused by RSV in the elderly.[5] Currently, there is no effective vaccine available for RSV, and treatment is limited to supportive care. Severe RSV-induced LRTIs are associated with and thought to be caused by severe pulmonary inflammation.

In addition, severe RSV infection during infancy has also been associated with increased risk for asthma development. There is substantial evidence indicating that children hospitalized with RSV-induced bronchiolitis are more likely to experience recurrent wheezing episodes for a prolonged period of time after recovery from this illness.[6-9]

Helminths infect approximately 3 billion people worldwide. It has long been proposed that infection with helminths could suppress the development of immune-mediated disease because in countries where their prevalence is high, the prevalence of asthma, allergy, and autoimmune conditions has been found to be correspondingly low.[10] In particular, intestinal helminths have been of major interest because of their ability to modulate host immune and inflammatory responses to foreign antigens,[11-16] and several clinical trials have been carried out or are underway assessing their utility as therapeutic agents in patients with inflammatory bowel disease, multiple sclerosis, and asthma.[17]

Helminth infections rarely occur in isolation, and coinfections are very common with varying effects, such as reduced pathogen control and increased disease, as reported for HIV infection and tuberculosis.[18-21] Recent experimental models in mice report reactivation of systemic latent γ-herpesvirus and reduced control of enteric norovirus replication,[22,23] indicating that in these systems helminth infection suppresses antiviral immunity, resulting in increased viral replication. However, the effect of helminth infection on respiratory viruses is not well understood. Clinical data are lacking, but mouse models suggest reduced influenza-induced pathology in helminth coinfection.[24,25]

Here we investigated whether infection with the strictly enteric murine helminth *Heligmosomoides polygyrus* would change the course of disease and inflammation during RSV infection. This study demonstrates protective effects of helminth infection on RSV infection and reveals a novel mechanism of type I interferon induction by enteric helminth infection at a site distant from the gut.

METHODS

Animals

BALB/c, C57BL/6, IL-4 receptor α *(Il4ra)*$^{-/-}$,[26] recombination-activating gene 1 *(Rag1)*$^{-/-}$,[27] IL-33 receptor *(Il33r)*$^{-/-}$ (BALB/c background), *Ifnar1*$^{-/-}$,[28] and *Camp*$^{-/-}$[29] (bred to congenicity on a C57BL/6J Ola Hsd background) mice were bred in house at the University of Edinburgh. Germ-free BALB/c mice were obtained from the Clean Mouse Facility, University of Bern (Bern, Switzerland), and compared with specific pathogen-free (SPF) BALB/c mice from Charles River Breeding Laboratories (l'Arbresle Cedex, France). Six- to 12-week-old female mice were infected by means of oral gavage with 200 stage 3 *H polygyrus* larvae. Ten days later, mice were intranasally infected with RSV or mock infected with UV-inactivated respiratory syncytial virus (UV-RSV; standard coinfection protocol).

Parasites, parasite products, and viral stocks

Parasites were maintained, as previously described.[30] For some experiments stage 3 *H polygyrus* larvae were irradiated at 100, 200, or 300 Gy by using a GSR-C1 irradiator at a rate of 6.2 Gy/min before administration by oral gavage. Axenic *H polygyrus* larvae were produced, as previously described.[31] Plaque-purified human RSV (Strain A2; ATCC, Manassas, Va) was grown in Hep-2 cells, as previously described.[32]

Whole-body plethysmography

Baseline respiratory effort was assessed in individual mice by using whole-body plethysmography (Buxco, Wilmington, NC). Mice were placed into individual chambers, and baseline measurements were recorded for 5 minutes. Enhanced pause values were recorded, averaged, and expressed as absolute values, as previously described.[33]

RSV immunoplaque assay

RSV titers were assessed, as previously described,[34] in lung homogenates by means of titration on HEp-2 cell monolayers in 96-well, flat-bottom plates. Twenty-four hours after infection, monolayers were washed, fixed with methanol, and incubated with biotin-conjugated goat anti-RSV antibody (Bio-rad, Watford, United Kingdom). Infected cells were detected with 3-amino-9-ethylcarbazole, and infectious units were enumerated by means of light microscopy.

Lung cell isolation and flow cytometry

Right lung lobes were excised, cut into small pieces, incubated on a shaker with collagenase A (0.23 mg/mL PBS; Sigma, St Louis, Mo) at 37°C for 45 minutes, and sheared through a 19-gauge needle. After red blood cell lysis (Sigma), the single-cell suspension was passed through a 40-μm cell strainer and stained with the viability dye eFluor 780 (eBioscience, Hatfield, United Kingdom). The following anti-mouse antibodies were used to phenotype lung immune cells: PDCA-1 (EBIO-927), Ly6G (RB6-C5), NKp46 (29A1.4), and B220 (RA3-6B2), all eFluor 450 conjugated (eBioscience, San Diego, Calif); Ly6C (AL-21) and CD8 (Ly-2), both fluorescein isothiocyanate conjugated (BD Biosciences, San Jose, Calif); CD11b (M1/70) and CD4 (RM4-5), both phycoerythrin conjugated (eBioscience); CD45 (30-F11), eFluor 605 Nanocrystal (NC605) conjugated (eBioscience); CD49B (DX5), CD19 (6D5; BioLegend, San Diego, Calif), and F480 (Cl:A3-1), all Alexa Fluor 647 conjugated (AbD Serotec, Bio-Rad Laboratories, Hercules, Calif); MHC class II (M5/114.15.2) and CD3 (145-2C11), both peridinin-chlorophyll-protein complex Cy5.5 conjugated (BioLegend); CD19 (EBIO1D3), CD3 (17A2; eBioscience), and Ly6G (1AB), all Alexa Fluor 700 conjugated (BD Biosciences); and CD11c (N418), phycoerythrin-Cy7 conjugated (eBioscience). Isotype control antibodies were used on pooled samples. Cells