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IFN Therapy in Airway Disease: Is Prophylaxis a New Approach in Exacerbation Prevention?

It is widely accepted that respiratory virus infections precipitate the great majority of acute asthma attacks (exacerbations) in all age groups (1). Evidence that viruses precipitate the great majority of acute exacerbations of chronic obstructive pulmonary disease (COPD) is also strong, in that the great majority of patients report colds before onset of exacerbations (2), colds are powerful predictors of exacerbation risk and severity (3), viruses are detected in exacerbations at rates up to 67% (4), it is increasingly recognized that many bacterial exacerbations result from secondary bacterial infection after initial virus infection (5, 6), and experimental virus infection induces exacerbation in ∼95% of infected volunteers with COPD (7).

Although not found in all studies (8), there is now abundant evidence that asthma is frequently accompanied by broadly impaired antiviral immunity in both adults (9–15) and children (16–18). Most of these studies report deficiencies in IFN-β and IFN-λ induction by virus infection of bronchial epithelial cells (bronchial epithelial cells do not produce IFN-α), as well as deficiencies in IFN-α, IFN-β, and IFN-λ induction by virus infection of macrophages/dendritic cells. These data clearly implicate IFN deficiency in the pathogenesis of asthma attacks. This interpretation is also strongly supported by the recent elegant demonstration that low IFN response gene expression in children with asthma strongly predicts future exacerbation risk (19).

Deficient virus-induced IFN responses have also been reported in BAL cells (7) and primary bronchial epithelial cells (PBECs) in COPD (20). Rhinovirus challenge studies in COPD have confirmed that increased virus replication is observed in both upper and lower respiratory tracts *in vivo* (7, 21).

These data have generated great interest in the potential of exogenous IFN therapy as an acute interventional treatment to prevent early symptomatic colds progressing to virus-induced asthma and COPD exacerbations. Djukanović and colleagues investigated the effect of inhaled IFN-β/placebo treatment for 14 days, initiated within 24 hours of diary-verified cold/influenza symptoms, in 134 patients with British Thoracic Society step 2–5 asthma with a history of at least one cold-related asthma exacerbation requiring oral corticosteroids and/or antibiotics in the last 24 months (22). There was no significant effect on the primary endpoint of asthma symptoms in the 7 days after treatment initiation; however, it should be noted that the colds did not result in a clinically meaningful worsening of asthma symptoms in the placebo-treated subjects; thus there was likely no clinically meaningful worsening of asthma symptoms for the IFN-β treatment to impact upon (22). There was a significant effect of IFN-β on the secondary endpoint of morning peak flow (mean difference, 19.5 L/min; $P = 0.03$), on CCL4 levels in sputum supernatants ($P = 0.035$), and on induction of antiviral activity in the lung (increased sputum ISG [IFN-stimulated gene] *OAS1* and *MX1* expression; $P = 0.0003$ and 0.0001).

In a preplanned subgroup analysis of 54 subjects with British Thoracic Society step 4–5 asthma, in whom colds did result in clinically meaningful worsening of asthma symptoms in the placebo group, there was a clinically meaningful and statistically significant improvement in symptoms ($P = 0.004$), and the percentage of patients with clinically meaningful worsening of asthma symptoms was lower for IFN-β (17%) compared with placebo (50%; $P = 0.012$). The improvement with treatment in morning peak flow was also greater (mean difference, 31.4 L/min; $P = 0.03$). Five patients receiving placebo, but only one receiving IFN-β, required oral corticosteroids/antibiotics.

In the INEXAS (A Study in Asthma Patients to Evaluate Efficacy, Safety and Tolerability of 14 Days Once Daily Inhaled Interferon Beta-1a After the Onset of Symptoms of an Upper Respiratory Tract Infection) phase 2 trial, 121 subjects with Global Initiative for Asthma step 4–5 asthma, with ⩾2 cold-related severe exacerbations in the last 2 years, were randomly assigned to inhaled IFN-β/placebo treatment for 14 days within 48 hours of cold/influenza symptoms (23). The primary outcome, severe exacerbations, was unexpectedly rare, with only 7 and 5 patients in the IFN and placebo groups, respectively, resulting in the trial being stopped early. As in the previous study, there was a significant improvement in morning peak flow with IFN-β ($P = 0.01$).

These studies together indicate that IFN-β-treated asthma exacerbations did show some evidence of efficacy, especially in subjects in whom clinically meaningful worsening of asthma symptoms occurred after colds. However, both studies suffered

Supported by European Research Council Advanced grant 788575, Asthma UK Centre grant AUK-BC-2015-01, and BBSRC/MRC Systems Immunology grant MR/L012693/1. The author is a National Institute for Health Research Emeritus Senior Investigator and the Asthma UK Clinical Chair.

Originally Published in Press as DOI: 10.1164/rccm.201909-1850ED on October 2, 2019

from better-than-expected outcomes after colds, likely a result of subjects increasing their inhaled steroid therapy as a consequence of taking part in clinical trials.

A notable finding in both studies was that peak worsening of asthma endpoints was observed at randomization, suggesting that treatment was started relatively late after cold onset, when asthma exacerbation was already maximal. Thus, earlier intervention very soon after cold symptom onset and before peak of asthma worsening is likely to be essential for on-demand IFN-β therapy to be effective.

As inhaled IFN-β was well tolerated, an alternative approach might be to consider prophylaxis, rather than intervention. In this issue of the *Journal*, Watson and colleagues (pp. 83–94) studied the dynamics of IFN responses to help inform future clinical trial design (24). Monocyte-derived macrophages (MDMs) differentiated from peripheral blood mononuclear cells and alveolar macrophages, and PBECs isolated from healthy control individuals and patients with COPD were infected with influenza virus before or after IFN-β stimulation. Infection was assessed by percentage of influenza A virus NP1 (nucleoprotein 1)-positive cells and by viral RNA load in culture supernatants by quantitative PCR. Treatment of MDMs with 50 IU/ml IFN-β 2 hours after infection had no effect on influenza infection at 24 hours, and a similar lack of effect of IFN-β 2 hours after infection was reported with doses of IFN-β up to 2,000 IU/ml.

The researchers then modeled prophylaxis by studying the effect of IFN-β added 16 hours before infection and found that this reduced frequency of $NP1^+$ cells by 85% and viral RNA in supernatants ~20-fold.

The authors then studied the dynamics of IFN-β induction of IFNs and ISGs in MDMs, and surprisingly found no induction of IFNs, although they reported only a single dose of IFN-β (50 IU/ml) at a single point after stimulation (not stated), in only $n = 3$ cultures, with other data "not shown." They did, however, report significant induction of the ISGs *MX1*, *OAS1*, *DDX58*, *CXCL10*, *IFIT1*, *ISG15*, and *RSAD2* 8–24 hours after stimulation with 50 IU/ml IFN-β.

They then investigated the duration of the IFN-β response by incubating MDMs with IFN-β for 16 hours and then removing the IFN-β and subsequently culturing cells for up to 2 weeks before measurement of ISG expression or assessment of antiviral activity. They report statistically significant antiviral activity out to 1 week after removal of IFN-β; however, the effects appear markedly diminished beyond 48 hours after IFN removal, and ISG induction also appears to wane beyond 48–72 hours after removal. In similar experiments in PBECs, ISG induction had largely waned at points beyond 24 hours after IFN-β removal.

The authors then assessed proinflammatory potential of IFN-β by measuring GM-CSF (granulocyte–macrophage colony–stimulating factor), TSLP, IL-25, IL-33, IL-1β, IL-6, TNF, CCL5, CCL17, and CCL22 concentrations in supernatants from uninfected MDMs stimulated with 50 IU/ml IFN-β for from 24 hours to 2 weeks. No induction of any of the above mediators was observed.

Next, the authors investigated whether repeated doses of IFN-β could desensitize macrophages to IFN-β treatment by chronically stimulating MDMs with 50 IU/ml IFN-β for up to 3 weeks (replacing IFN-β twice weekly), followed by a final administration of IFN-β 16 hours before influenza infection. No reduction in antiviral activity was observed with chronic dosing, suggesting desensitization did not occur.

Further experiments were performed to determine whether IFN-β modulates influenza infection in lung-derived macrophages and PBECs. Influenza infection of lung macrophages was limited with only very modest increased cellular NP1 expression and viral RNA in supernatants at 24 hours after infection, and suppression by 16 hours pretreatment with IFN-β, although statistically significant, was also modest. Infection of PBECs was more robust, and suppression by 16 hours pretreatment with IFN-β also robust. Treatment 2 hours after infection was ineffective in both cell types.

The duration of IFN-β antiviral activity in PBECs was then investigated by incubating PBECs with IFN-β for 16 hours and then removing the IFN-β and subsequently culturing the PBECs (with no further treatment) for up to a further week before influenza infection. IFN-β maintained the capacity to significantly inhibit influenza infection of PBECs up to 72 hours after its removal; however, there was a gradual reduction in antiviral activity from as early as 24 hours, with antiviral activity approximately halved at 48 hours after removal. A similar time course was present for IFN-β-mediated inhibition of influenza-induction of IL-1β, and for ISG induction by IFN-β in the absence of infection. Duration of activity of IFN-β was no different in PBECs or in MDMs from healthy volunteers compared with those from patients with COPD.

The authors finally report that IFN-β did not induce inflammatory mediators from PBECs, including GM-CSF, IL-1β, TNF, and IL-6, and that CCL5, CCL17, CCL22, IL-25, IL-33, and TSLP were below assay detection limits.

The authors are to be congratulated for performing this extensive set of investigations in primary human cells, and for identifying a potential new approach to IFN therapy: using prophylaxis to prevent virus-induced exacerbations instead of acute intervention. Depending on which assay one looks at, the activity of IFN-β seemed to wane starting from 24 hours after dosing, but significant effects were still detectable several days after dosing, raising the possibility that dosing intervals greater than daily might have potential to be effective. Further work is certainly needed to extend these observations with a greater body of evidence in PBECs, with rhinoviruses (as by far the most common precipitant), and with IFN-λ (as the most abundant IFN induced in PBEC). We thank the authors for extending our knowledge in relation to IFN therapy in airway disease and for hopefully stimulating further work on this important subject, including further clinical trials of this approach. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Promising Advances for Imaging Lung Macrophage Recruitment

Pulmonary hypertension (PH) contributes significantly to morbidity and mortality and has no curative therapies. Patients with World Health Organization group I pulmonary arterial hypertension (PAH) have improved survival as a result of targeted treatments, but 5-year survival remains low at 57% to 61% (1, 2), with a significant proportion ultimately requiring lung transplantation (3–5). Inflammation is being increasingly recognized as an important contributor to PAH development and progression (6). Therapies that reduce lung macrophage recruitment also reduce PH in animal models, further demonstrating the relevance of macrophages in PAH pathogenesis (7). Therefore, noninvasive biomarkers of lung macrophage recruitment and activity could help demonstrate the efficacy of macrophage-targeted therapies as well as enable investigations to understand how macrophages contribute to PAH development. Such biomarkers would also be applicable more broadly in multiple different lung diseases, including chronic obstructive pulmonary

Supported by Wil B. Nelp, M.D., Endowed Professorship in Nuclear Medicine.

Originally Published in Press as DOI: 10.1164/rccm.201907-1455ED on August 5, 2019