A deficient immune response to viral infections in children predicts later asthma persistence

Simonetta Baraldo[1], PhD, Marco Contoli[2], MD, PhD, Matteo Bonato[1] MD, Deborah Snijders[3], MD, Davide Biondini[1] MD, Erica Bazzan, PhD, Manuel G. Cosio[1,4], MD, Angelo Barbato[3], MD, Alberto Papi[2*], MD, Marina Saetta[1]*, MD.

[1]Dept. of Cardiac, Thoracic and Vascular Sciences, Respiratory Diseases Clinic, University of Padova, Padova, Italy; [2]Dept. of Medical Sciences, Section of Respiratory Diseases, University of Ferrara, Ferrara, Italy; [3]Dept. of Woman and Child Health, University of Padova, Padova, Italy; [4] Meakins-Christie Laboratories, Respiratory Division, McGill University, Montreal, Canada.

Correspondence to: Marina Saetta, Dept. of Cardiac, Thoracic and Vascular Sciences, University of Padova, Via Giustiniani 3, 35128 Padova, Italy. e-mail: marina.saetta@unipd.it; tel +39 0498218515; fax + 390498213110.

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*These authors equally contributed

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To the editor

Rhinovirus infection is the most frequent cause of asthma exacerbations in children, and respiratory viral infections in early life have been associated with increased risk of asthma later on (1). However, the mechanisms driving this association are incompletely understood. We recently showed that deficient antiviral immune responses by bronchial epithelium, with decreased rhinovirus-induced IFNs production and increased viral replication, are already present in preschool asthmatic children (with or without atopy) and in atopic children without asthma (2). Our original cohort of 34 children was prospectively followed for 8 years in order to investigate if the impaired rhinovirus-induced interferon production seen in the original population could be a risk factor for the development of asthma later in life (as diagnosed by a respiratory pediatrician blinded to antiviral response data).

Our results seem to confirm this possibility. Analyses of epithelial IFN production and viral replication along with epithelial destruction and eosinophil counts in bronchial biopsies were performed as previously reported (2). Follow-up data were available for 27 children: 62% of children with asthma at baseline remained asthmatic at follow-up, while 21% of children with no asthma at baseline developed asthma. Children with asthma at follow-up, when compared to non-asthmatics, had lower IFNλ and IFNβ and increased viral replication at baseline (Table 1, Figure 1). At baseline, the deficient IFN production was associated to the percentage of destroyed epithelium in bronchial biopsies ($r=0.68, p<0.05$). After 8 years we found an association between impaired IFN at baseline and frequency of asthmatic symptoms and antibiotics use during follow-up. Notably, children with no asthma at baseline, who later became asthmatics (Figure 1, open circles), also had significantly lower IFNs and increased viral replication compared to those who remained non-asthmatics (IFNλ:2.9±0.1 vs 3.8± 0.9; IFNβ: 2.9±0.5 vs 3.9±1; RV: 5.7±0.9 vs 4.8±0.8 log10copies/μgRNA);all $p<0.05$]. These findings confirm previous data showing that an abnormal immune response in asymptomatic infants would promote persistent airway inflammation and asthma later on (3,4). Altogether these data support the concept that an inappropriate response to infectious pathogens, either viral or bacterial, may not only be important for the persistence of symptoms but even precede the development of asthma.

A strength of our study is that we investigated IFNs production directly from airway epithelial cells, the site where infections occur, which allowed us to correlate the epithelial functional changes with the structural changes in bronchial biopsies, in particular epithelial damage. Epithelial damage is now increasingly recognized as a crucial hallmark of asthma pathogenesis (5), not only by the newly described deficient immune function,

but also by its fragility and impaired barrier function that would favor the passage of allergens and pathogens into the airways.

In our study, the decreased production of interferons by epithelial cells was also associated with the extent of airway eosinophilia (r=-0.76,p=0.001), as part of the Th2 pattern of inflammation. The mechanisms linking Th2-induced eosinophilia with viral infections and IFN responses are complex. On one side, mice with congenital IFN deficiency develop airway eosinophilia and T_H2 cytokines when challenged with viruses (6); on the other, T_H2-induced airway eosinophilia inhibits IFNs and impairs antiviral responses (7). These data imply a reciprocal regulation between antiviral responses and eosinophils in the airways with relevant clinical consequences: impaired IFNs responses to viral infections would promote T_H2 inflammation, and T_H2-eosinophilic inflammation may, in turn, reduce the ability to respond to viruses.

In addition, when stratified by IgE levels (above or below the median) we found that children with high IgE produced less IFNs [IFNλ:3.1±0.5 vs 4.1±1, p=0.007; IFNβ:3.4±1 vs 4.1±1 log10copies/μgRNA, p=0.05). IgE levels were correlated to peripheral blood eosinophils (r=0.66; p=0.0002) and biopsy eosinophils (r=0.43; p=0.05). These results indicate that, along with eosinophils, IgE levels were related to an impaired IFN production by epithelial cells, supporting the importance of a negative modulation between IgE and IFN not only in systemic antiviral responses (8) but also in local responses, though the mechanisms are yet undetermined.

The relative contributions of respiratory viral infections *vs* atopy in asthma pathogenesis are still debated. It has long been recognized that risk for asthma in childhood tracks with atopy. Emerging evidence shows that early wheezing with respiratory infections is an important risk factor for the development of asthma, and the effect of infections may even overcome the influence of sensitization at early time points (1). Thus, it has been suggested that an impaired immune response of the airway epithelium may be the primary disturbance in asthma, which may even anticipate atopy. Conversely, it is also possible that T_H2 inflammation may be the initiating event inducing an impairment of IFNs production with poor response to viruses and worsening of asthma. In this context, recent clinical evidence supports the hypothesis of an early effect of T_H2 inflammation on viral-induced exacerbations: preventive treatment of children with anti-IgE or ICS before return to school reduced viral-induced asthma exacerbations during the fall, an effect that was associated with increased IFN production (9).

It should be highlighted that the complex design of our study, requiring bronchial brushing and biopsies, may imply some limitations since for ethical constraints we had to study children undergoing a clinically indicated bronchoscopy. However the indications for bronchoscopy did not differ between asthmatic and non-asthmatic children which, together with similar results recently obtained in non-bronchoscopic bronchial brushings (10), supports the validity of our results. We should acknowledge that there was a certain degree of overlap in IFNs

responses between asthma and non-asthma groups. However, in biological experiments, an overlap of this kind is usually expected. Despite these unavoidable biases, the conclusions we reached, suggesting that impaired immune responses to infective agents in children are linked to the presence of asthma later in life, are in agreement with published epidemiological data (3,4) and offer a plausible mechanism for this association.

In conclusion, our study suggests that a deficient epithelial interferon response to rhinovirus in early childhood related to epithelial damage, in combination with T_H2 inflammation and atopy, which are known depressors of IFN production, are key factors in determining the natural history of childhood asthma.

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Figure Legend

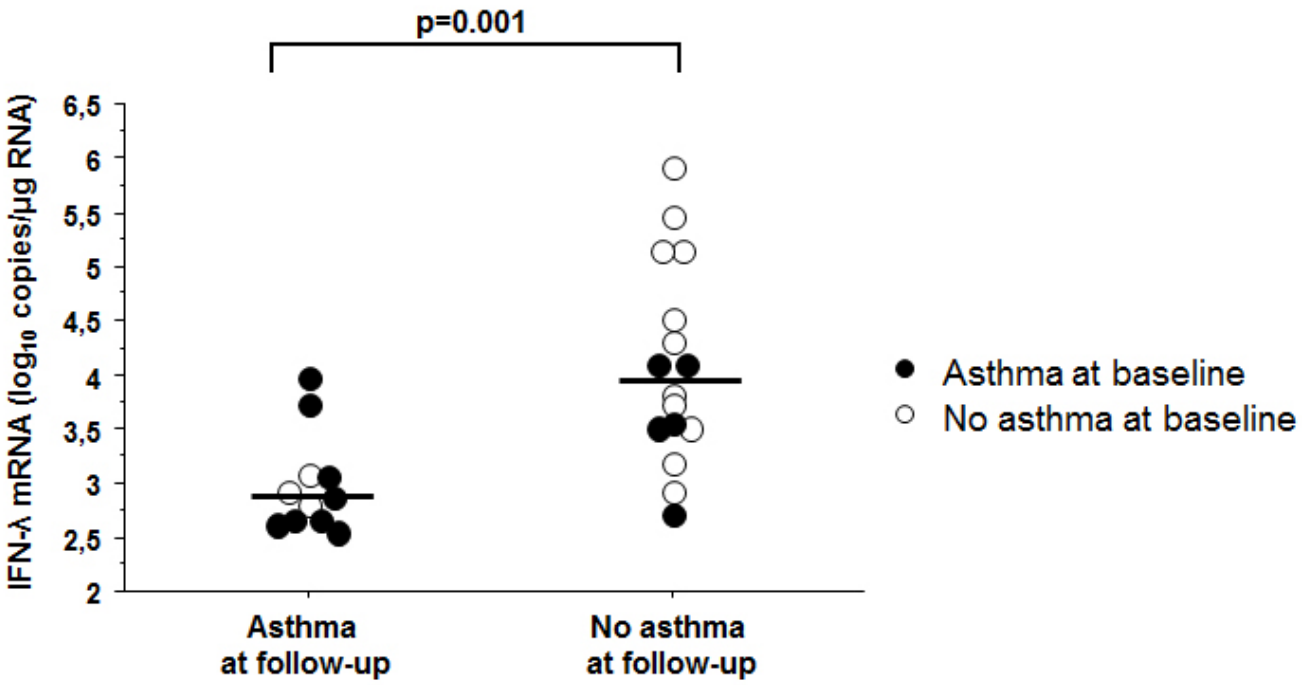
Figure 1 IFN-λ production at baseline in relation to asthma or no asthma at follow-up

Rhinovirus (RV16)-induced IFN-λ mRNA levels at baseline were decreased in children with asthma at follow-up compared to those with no asthma at follow-up. Closed circles represent children with asthma at baseline, open circles represent children without asthma at baseline. Comparison between groups was evaluated by Student's t-tests.

Table 1. Characteristics of the cohort in relation to asthma or no-asthma at follow-up

	Asthma at follow-up (n=11)	No asthma at follow-up (n=16)
BASELINE CHARACTERSTICS		
Age at baseline (yrs)	5.7±3	5±3
Total IgE (U/ml)	517±902†	187±537
Eosinophils in blood (cells*10^9/L)	0.50±0.48	0.26±0.18
Eosinophils in biopsies (cells/mm^2)	86 (9-180)†	11(0-304)
IFN-λ mRNA (log_{10} copies/µg RNA)	3.0 ± 0.46 *	4.1 ± 0.93
IFN-β mRNA (log_{10} copies/µg RNA)	3.0 ± 0.40 †	4.2 ± 0.98
IFN-β protein (pg/ml)	1.6±0.2 †	2.6±1.2
Viral RNA (log_{10} copies/µg RNA)	5 ± 0.68 †	4.5 ± 0.82
FOLLOW-UP DATA		
Duration of follow up (yrs)	7.6±0.7	7.8±0.5
Age at follow-up (yrs)	13.3±3	12.8±2.9
FEV1/FVC (%)	84±10	90±8
FEV1 (% pred)	84±10	85±11
FeNO (ppb)	17 (7-137)	14 (6-54)
Regular inhaled steroids+oral courses n. of children (%)	1 (9%)	0 (0%)
Regular inhaled steroids n.of children (%)	4 (36%)*	0 (0%)
Intermittent inhaled steroids n.of children (%)	4 (36%)*	0 (0%)
Frequency of asthma symptoms (score) #	2 [0-5]*	0
Antibiotic courses in the last year (n.)	1.7±1.8†	0.4±0.8

Data are expressed as mean ± SD or as median (range); comparisons between groups were evaluated with either χ^2, Student's t-tests or Mann-Whitney-U-test. † $p<0.05$, * $p<0.005$. # Frequency Scale (0 = no symptoms; 1 = less than 1 episode/month; 2 = 1 episode/month; 3 = 2-3 episodes/month; 4 = 1 episode/week; 5 = more than 1 episode/week; 6 = daily episodes).


p=0.001
IFN-λ mRNA (log10 copies/µg RNA)
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
Asthma at follow-up
No asthma at follow-up
Asthma at baseline
No asthma at baseline

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