

## **Reduced Type-I Interferon by Plasmacytoid Dendritic Cells and Asthma in School-Aged Children**

### Author

Coenen, Isabelle, Jones, Anya C, White, Alice A, Takashima, Mari, Lee, Wen Ray, Wong, Matthew D, Vilcins, Dwan, Kadolsky, Ulrich, Cheema, Ali Sadiq, Saxena, Alka, Bosco, Anthony, Grimwood, Keith, Sly, Peter D, Strickland, Deborah H, Leffler, Jonatan

### Published

2025

### Journal Title

Allergy

### Version

Version of Record (VoR)

### DOI

[10.1111/all.70005](https://doi.org/10.1111/all.70005)

### Rights statement

© 2025 The Author(s). Allergy published by European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

### Downloaded from

<https://hdl.handle.net/10072/440250>

### Griffith Research Online

<https://research-repository.griffith.edu.au>

## ORIGINAL ARTICLE OPEN ACCESS

Basic and Translational Allergy Immunology

# Reduced Type-I Interferon by Plasmacytoid Dendritic Cells and Asthma in School-Aged Children

Isabelle Coenen<sup>1</sup> | Anya C. Jones<sup>1,2</sup> | Alice A. White<sup>3</sup> | Mari Takashima<sup>4</sup> | Wen Ray Lee<sup>4</sup> | Matthew D. Wong<sup>4</sup> | Dwan Vilcins<sup>4</sup> | Ulrich Kadolsky<sup>5</sup> | Ali Sadiq Cheema<sup>5</sup> | Alka Saxena<sup>5</sup> | Anthony Bosco<sup>2,6,7</sup> | Keith Grimwood<sup>8</sup> | Peter D. Sly<sup>4</sup> | Deborah H. Strickland<sup>1</sup> | Jonatan Leffler<sup>1</sup>

<sup>1</sup>Wal-Yan Respiratory Research Centre, The Kids Research Institute Australia, University of Western Australia, Perth, Western Australia, Australia | <sup>2</sup>INSIGENE Pty Ltd, Perth, Western Australia, Australia | <sup>3</sup>The Kids Research Institute Australia, University of Western Australia, Perth, Western Australia, Australia | <sup>4</sup>Child Health Research Centre, The University of Queensland, South Brisbane, Queensland, Australia | <sup>5</sup>Genomics WA, Perth, Western Australia, Australia | <sup>6</sup>Asthma and Airway Disease Research Center, University of Arizona, Tucson, Arizona, USA | <sup>7</sup>Department of Immunobiology, University of Arizona College of Medicine, Tucson, Arizona, USA | <sup>8</sup>School of Medicine and Dentistry, Griffith University, Gold Coast, Queensland, Australia

**Correspondence:** Jonatan Leffler ([jonatan.leffler@thekids.org.au](mailto:jonatan.leffler@thekids.org.au))**Received:** 17 February 2025 | **Revised:** 11 June 2025 | **Accepted:** 29 June 2025**Funding:** The study was funded by the Wal-yan Respiratory Research Centre, and J.L. is supported by a fellowship from the Stan Perron Charitable Foundation.**Keywords:** childhood asthma | IgE | interferon response | plasmacytoid dendritic cells

## ABSTRACT

**Background:** Allergic sensitization and reduced ability to respond to viral infections may contribute to virus-induced wheeze and asthma development in young children. Plasmacytoid dendritic cells (pDC) are rare immune cells that produce type I interferons (IFN-I) and play a key role in orchestrating immune responses against viruses.

**Objective:** To further evaluate the function of pDC in children with asthma.

**Methods:** This study was based on a subset of 71 children from the Early Life Lung Function (ELLF) cohort at the age of 7 years. As part of the ELLF study, participants were characterized for atopic sensitization, viral infection history, and lung function testing. pDC responses to a TLR7/8 agonist were assessed in the presence or absence of anti-IgE using an in vitro assay. Responses were evaluated utilizing flow cytometry, multiplexed cytokine assays, and transcriptional analysis of isolated pDC.

**Results:** pDC responses varied considerably across individuals, and those who responded with IFN-I following stimulation showed a lower proportion of asthma compared to those who responded with TNF-only. A TNF-only response was associated with increased atopy and reduced upregulation of IFN-associated genes. Anti-IgE stimulation reduced pDC activation, and the reduction was associated with baseline expression of the IgE receptor (FcεR1). A reduction in a gene module centralized around genes such as TPM2, LILRA4, and CLEC4C was also observed.

**Conclusion:** Together, these findings suggest that pDC responses are variable, associated with asthma, and appear influenced by environmental stimuli. This response thus appears to be an important aspect of asthma pathology in children.

## 1 | Introduction

In young children, wheezing during a viral respiratory infection is common and affects almost 50% of children before

the age of 5 years [1]. However, some children experience recurrent episodes of wheeze, and a subset of these are subsequently diagnosed with asthma. Impaired innate immune responses to viruses [2], atopic sensitization [3] and repeated

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2025 The Author(s). *Allergy* published by European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd.

infections [4] leading to fever [5] appear to be associated with asthma development [6]. However, the contribution of each of these factors remains unclear and controversial [7]. A current hypothesis suggests that atopic sensitization negatively impacts anti-viral responses, which in turn leads to repeated infections and increased risk of asthma development [3].

Plasmacytoid dendritic cells (pDC) are a rare immune cell subset and constitute <0.5% of circulating immune cells [8]. They are major producers of type I interferons (IFN-I) and central to anti-viral responses, including rhinovirus infection [9]. An excessive Th2 response, elevated IgE serum levels, or IgE crosslinking all negatively impact IFN-I responses [10–12]. On pDC, the impact of IgE is reversible using anti-IgE therapies such as Omalizumab [13] that also reduced asthma exacerbations in a clinical setting [14, 15]. We and others have previously demonstrated the high affinity IgE receptor (FcεR1) is upregulated in children with atopic asthma compared to atopic children without asthma [16, 17]. We have also demonstrated reduced levels of pDC during the first years of life increased asthma risk at 5 years of age [18] and that early life interferon responses are decreased in children who developed asthma later in childhood [19]. Furthermore, we demonstrated that failure to mount interferon responses in pDC during wheezing episodes appears more common in children diagnosed with asthma compared to those with resolving wheeze [20]. Although significant progress has been made in our understanding of asthma pathology, the molecular mechanisms integrating IgE sensitization, virus responses, and asthma remain unclear.

In the current study, we leveraged the rich clinical history of the Early Life Lung Function (ELLF) cohort [21] and employed short-term in vitro cultures to evaluate the functional response of pDC and the impact of IgE crosslinking on this response. We observed at 7 years of age two distinct phenotypical pDC responses following TLR7/8 stimulation, where a response defined by lack of IFN production was associated with current asthma and elevated atopy. In vitro IgE crosslinking resulted in decreased pDC activation and reduced expression of a gene module with hub-genes associated with IFN regulation.

## 2 | Methods

### 2.1 | Study Participants

The ELLF study assessed respiratory outcomes in children by conducting annual reviews between ages 3–7 years, including standardized respiratory health questionnaires and physical examinations [22]. These children were originally part of the Observational Research in Childhood Infectious Diseases (ORChID) study [23, 24] (Table S1), an unselected community-based cohort in Brisbane, Australia, which followed 158 healthy term-born infants from birth until their second birthday. After informed consent was obtained, parents collected weekly nasal swabs and recorded daily symptom diaries. Of the 158 children enrolled in the original ORChID cohort, 84 (53.2%) children participated in the ELLF extension study and provided asthma

outcome data at ages 5–7 years. Detailed descriptions of both studies have been published previously [25–27] and have been approved by the local ethics committee. Of the 84 ELLF participants, 71 (84.5%) provided peripheral blood samples for this study (Table S1). In this study, current asthma was defined as experiencing wheezing or taking beta-2 agonist or inhaled corticosteroid asthma medication during the last 2 years. Atopy was defined as returning a positive skin prick test to one of the following allergens: cat, house dust mite, mixed mold, or bahia grass pollen.

### 2.2 | Cell Thawing and Culture

Peripheral blood mononuclear cells (PBMCs) were thawed at 37°C in RPMI supplemented with 10% heat-inactivated fetal calf serum (Hi-FCS). Cells were washed, and viability was determined using trypan blue exclusion. For each sample, a portion of cells was allocated for ex vivo staining (see below) and the remaining cells resuspended in RPMI with 10% Hi-FCS and 50 μM 2-Mercaptoethanol. Following this,  $0.5 \times 10^6$  cells were transferred per well into a 96-well plate and cultured with 10 μg/mL of the TLR7/8 agonist Resiquimod (R848, Invivogen) or as an unstimulated, cell media-only control. For some cultures, cells were pre-incubated with 4 μg/mL rabbit anti-IgE (Bethyl Laboratories) for 1 h at 37°C and 5% CO<sub>2</sub> prior to 1 h stimulation with R848, after which, 5 μg/mL of the Golgi block, Brefeldin A (Biolegend) was added, and cells were incubated for an additional 16 h. For a subset ( $n = 23$ ) of samples (Table S2), cultures were conducted in duplicate with no Brefeldin A added to the second well. These samples were utilized for flow cytometry-assisted cell sorting for transcriptomic analysis, as detailed in the [Supporting Information](#), as well as cytokine analysis using the Bio-Plex Pro human Cytokine 17-plex Assay (BioRad) and the VeriKine human Interferon-Alpha ELISA kit TCM (PBL Assay Science) on thawed cell culture supernatant according to the manufacturer's instructions or as per previously [28]. Lower limits of detection for the Bio-Plex assay are displayed in Table S3.

### 2.3 | Statistics

For comparisons of continuous variables across two groups, such as for children with and without asthma or children with an IFN<sup>hi</sup> versus IFN<sup>low</sup> pDC response, the Mann-Whitney *U* test was used. For paired analysis, following in vitro stimulation, a Wilcoxon matched-pair test was utilized. Hierarchical clustering, using the Ward method, was utilized to identify pDC response clusters, and Fisher exact test or chi-square tests were used to identify associations across nominal variables. Adjustment for multiple comparisons was made where relevant using a two-stage step-up method [29]. For evaluation of potential confounders, a nominal logistic regression was utilized. Comparison of season-adjusted time to first virus infection was evaluated using a parametric survival model. Correlations between continuous variables were calculated using Spearman's correlation. All statistical analysis was performed using JMP 17.2.0 software (SAS). Data were visualized by Prism 10.4.1 software (GraphPad).

**TABLE 1** | Table of subset of ELLF cohort utilized in this study.

Clinical parameter	Current asthma (n = 26)	No current asthma (n = 45)	p
Age (year), median (range)	7.03 (7.0–8.0)	7.05 (7.0–7.8)	0.19
Male, N (%)	12 (46.2%)	21 (46.7%)	0.97
Vaginal delivery, N (%)	16 (61.5%)	30 (66.7%)	0.66
Current smoking in Family, N (%)	0 (0%)	8 (17.8%)	0.02
Attending center-based care, N (%)	17 (65.4%)	29 (64.4%)	0.94
Family history of Asthma, N (%)	15 (57.7%)	24 (53.3%)	0.72
History of wheeze, N (%)	23 (88.5%)	20 (44.4%)	<0.001
Current Atopy, N (%)	9 (36.0%)	12 (26.6%)	0.41
Current Eczema, N (%) <sup>a</sup>	9 (34.6%)	12 (26.7%)	
Confirmed HRV infection < 2 year, N (%)	26 (100%)	44 (97.8%)	0.44
Confirmed RSV infection < 2 year, N (%)	14 (53.85%)	27 (60.0%)	0.61
Season of peripheral blood collection			
Winter, N (%)	9 (34.6%)	9 (20.0%)	0.29
Spring, N (%)	6 (23.1%)	16 (35.6%)	
Summer, N (%)	8 (30.8%)	10 (22.2%)	
Fall, N (%)	3 (11.5%)	10 (22.2%)	

<sup>a</sup>Data missing from one individual.

### 3 | Results

#### 3.1 | Asthma-Specific Circulating Immune Cell Profile

To assess if children with asthma displayed a specific immunologic profile, immune cell abundance was evaluated ex vivo in 71 ELLF-cohort [25–27] participants at the age of 7 years (Table 1). Immune cell subsets were identified using two custom-made immune cell panels (Figure 1A,B, Figures S1 and S2) and abundances were plotted across the cohort (Figure 1C). Abundances were also compared between children with and without current asthma (Figure 1D). Children with asthma displayed an increased abundance of NK CD16<sup>hi</sup> cells and B Memory IgG<sup>+</sup> cells (Figure 1E) whereas the abundance of conventional DC type 2 (cDC2), CD4<sup>+</sup> T cells, and pDC were decreased (Figure 1F).

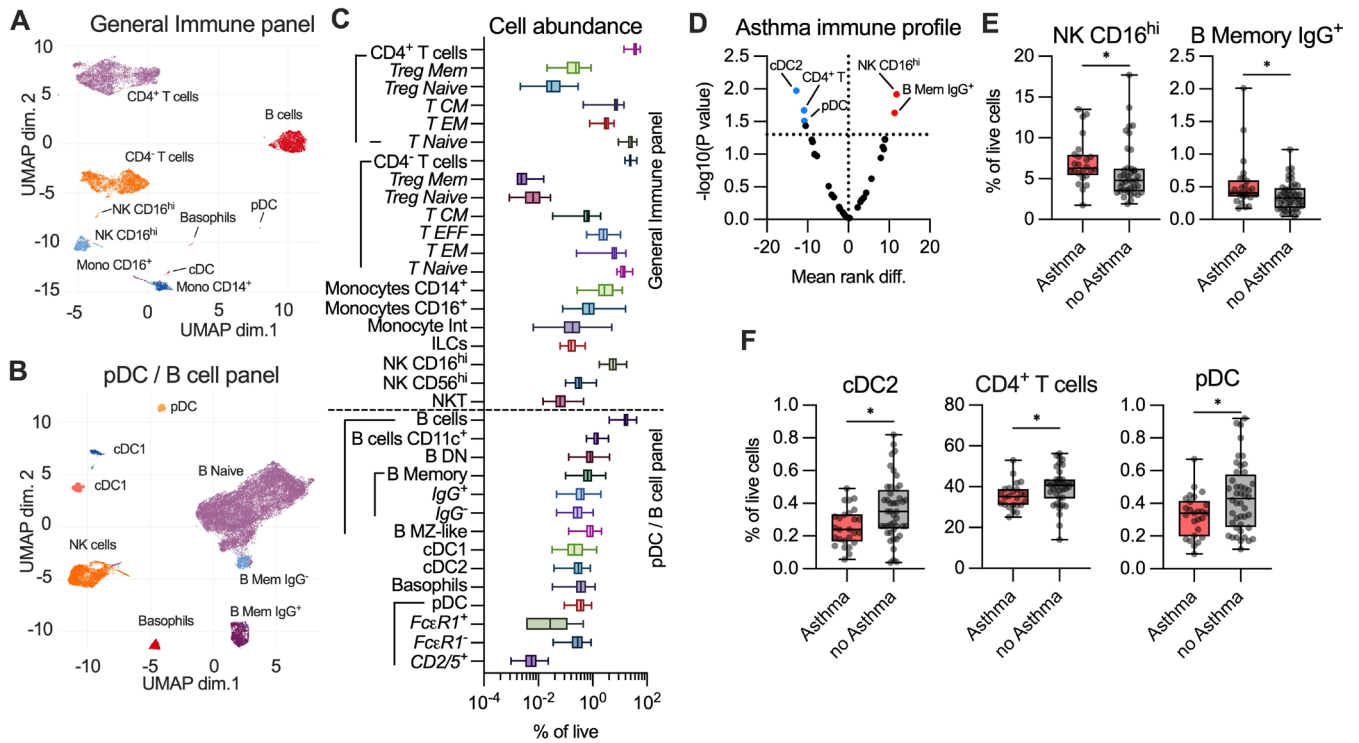
#### 3.2 | pDC Responses Associate With Asthma

To follow-up on our previous finding that viral response networks were decreased during an episode of wheezing in children who were later diagnosed with asthma [20], we evaluated pDC responses to R848 stimulation in vitro utilizing production of TNF, IFN $\alpha$  and IP-10 (CXCL10). These responses were manually gated (Figure S3) and three types of cytokine-producing pDC subsets were quantified: (i) TNF<sup>+</sup> [IFN<sup>-</sup>/IP-10<sup>-</sup>], (ii) TNF<sup>+</sup> [IFN<sup>+</sup>/IP-10<sup>+</sup>] and (iii) TNF<sup>-</sup> [IFN<sup>+</sup> or IP-10<sup>+</sup>]. The abundance of these subsets in each individual was used for hierarchical clustering, which generated three clusters: (a) IFN/IP-10 dominated, (b) TNF dominated, and (c) Low response. These were used to form two IFN<sup>hi</sup> and IFN<sup>low</sup> pDC response clusters (Figure 2A).

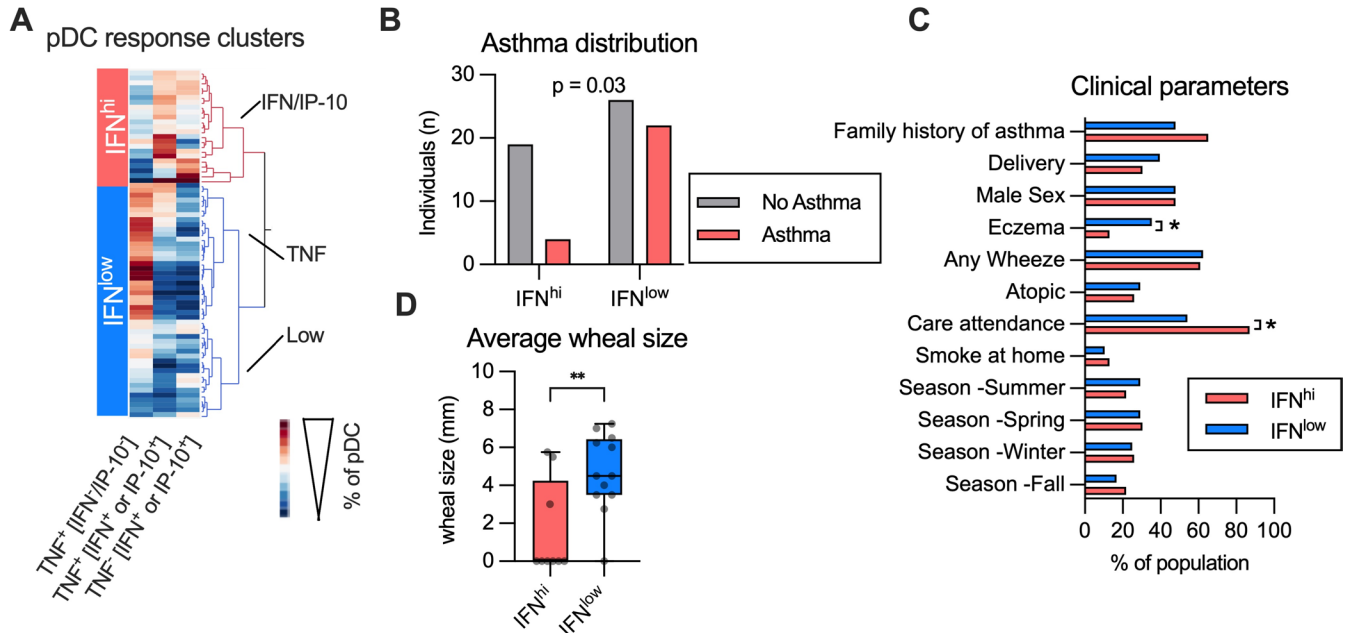
Comparing the proportion of children with current asthma in the two response clusters suggested an IFN<sup>hi</sup> response was associated with a reduced likelihood of asthma compared to individuals with an IFN<sup>low</sup> response (Figure 2B). This association remained significant after adjusting for current asthma therapies that may impact pDC responses (Figure S4A). The IFN<sup>hi</sup> and IFN<sup>low</sup> responders were also compared across other clinical parameters, identifying that IFN<sup>low</sup> individuals displayed increased rates of eczema, whilst they were less likely to have attended care during the last year (Figure 2C, Table S8). Care attendance was also independent of asthma status (Figure S4B–D). Of note, there was no difference in the prevalence of children who ever wheezed or had current atopy (Figure 2C). However, the IFN<sup>low</sup> group displayed significantly elevated average skin prick test wheal size compared to the IFN<sup>hi</sup> group (Figure 2D), suggesting a higher degree of sensitization in IFN<sup>low</sup> individuals compared to the IFN<sup>hi</sup> group. The association between asthma and IFN<sup>low</sup> responses remained significant after adjusting for wheal size (Figure S4E).

#### 3.3 | Limited Difference in Early-Life Viral Infection History Between IFN<sup>hi</sup> and IFN<sup>low</sup> Responding Individuals

IFN-I responses are essential for virus defense; to identify potential susceptibilities we compared the early-life history of virus infections in the two groups. We did not observe any difference in season-adjusted time-to-first infection of either respiratory syncytial virus (RSV) or human rhinovirus (HRV) infection following birth (Figure 3A,B). However, children in the IFN<sup>hi</sup> group had a higher proportion of HRV+ tests during the first



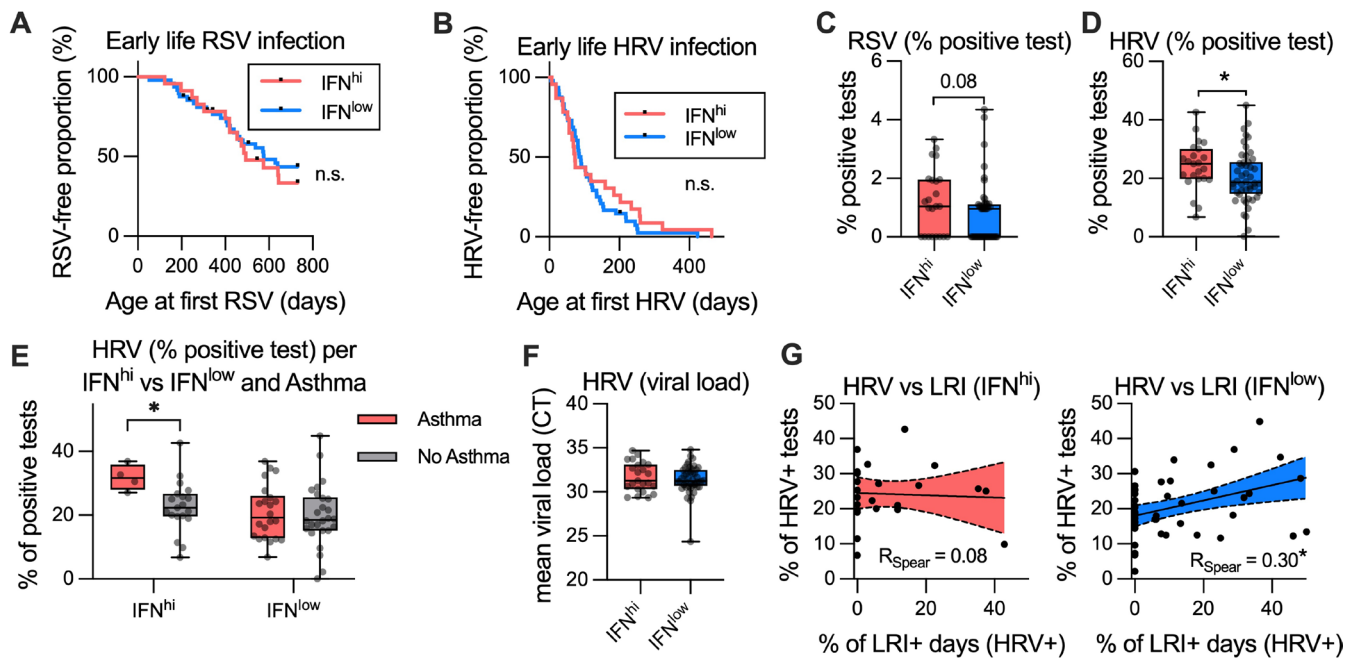
**FIGURE 1** | Immune cell abundance in children with asthma (A, B). Flow-SOM clusters projected onto a UMAP of immune cell populations identified using a general immune panel (A) or a pDC/B cell panel (B). (C) Cell abundance of relevant immune cell subsets from samples with ex vivo data available. (D) Abundance of immune cell subsets in children with asthma or with no asthma. (E, F) Immune cell subsets identified to be increased (E) or decreased (F) in children with asthma. The significance of the difference between children with asthma and with no asthma was calculated using the Mann-Whitney test;  $p$ -values are displayed as  $*p < 0.05$ .



**FIGURE 2** | pDC response to stimulation and association with asthma. (A) Clustering of pDC responses based on TNF, IFN $\alpha$ , and IP-10 expression following R848-stimulation. (B) Prevalence of asthma in the two main response clusters, IFN $^{hi}$  and IFN $^{low}$ . (C) Associations of pDC responses and clinical parameters. (D) Comparison of average wheal size in IFN $^{hi}$  and IFN $^{low}$  individuals. Statistical significance of differences was calculated using Fisher's Exact test for (B, C). The Mann-Whitney test was used for (D).  $p$ -values are displayed as a number or as  $*p < 0.05$ ,  $**p < 0.01$ .

2 years of life (Figure 3C,D). In particular, children in the IFN $^{hi}$  group with asthma at 6–7 years of age appeared with a high proportion of HRV+ tests (Figure 3E). In the same analysis, no

difference in mean viral load was observed between the two groups (Figure 3F). Interestingly, in the IFN $^{low}$  group, an increased proportion of positive HRV tests positively correlated



**FIGURE 3** | pDC response clusters and association to early life virus infection. (A, B). Time from birth to first detection of RSV (A) or HRV (B) in individuals with an IFN<sup>hi</sup> or IFN<sup>low</sup> response. (C, D). Proportion of RSV (C) and HRV (D) positive tests returned from weekly swabs in IFN<sup>hi</sup> or IFN<sup>low</sup> individuals. (E) Proportion of HRV positive tests in children with or without asthma split across IFN<sup>hi</sup> or IFN<sup>low</sup> responses. (F) Average C<sub>t</sub>-values during HRV+ swabs as a proxy for viral load in IFN<sup>hi</sup> and IFN<sup>low</sup> individuals. (G) Correlation between the proportion of positive HRV tests and the proportion of days with a lower respiratory tract infection (LRI) coinciding with HRV infection in children with IFN<sup>hi</sup> or IFN<sup>low</sup> responses. Statistical significance of differences in (A, B) was calculated using a parametric survival fit adjusted for the season of birth. In (C, F), a Mann–Whitney test was used, and Spearman’s correlation was used to evaluate associations in (G). *p*-values are displayed as \**p* < 0.05.

with the percentage of days with a lower respiratory tract infection (LRI), whereas in the IFN<sup>hi</sup> group, no such correlation was observed (Figure 3G).

### 3.4 | pDC Responses Are Associated With pDC Phenotype and T Cell Abundance

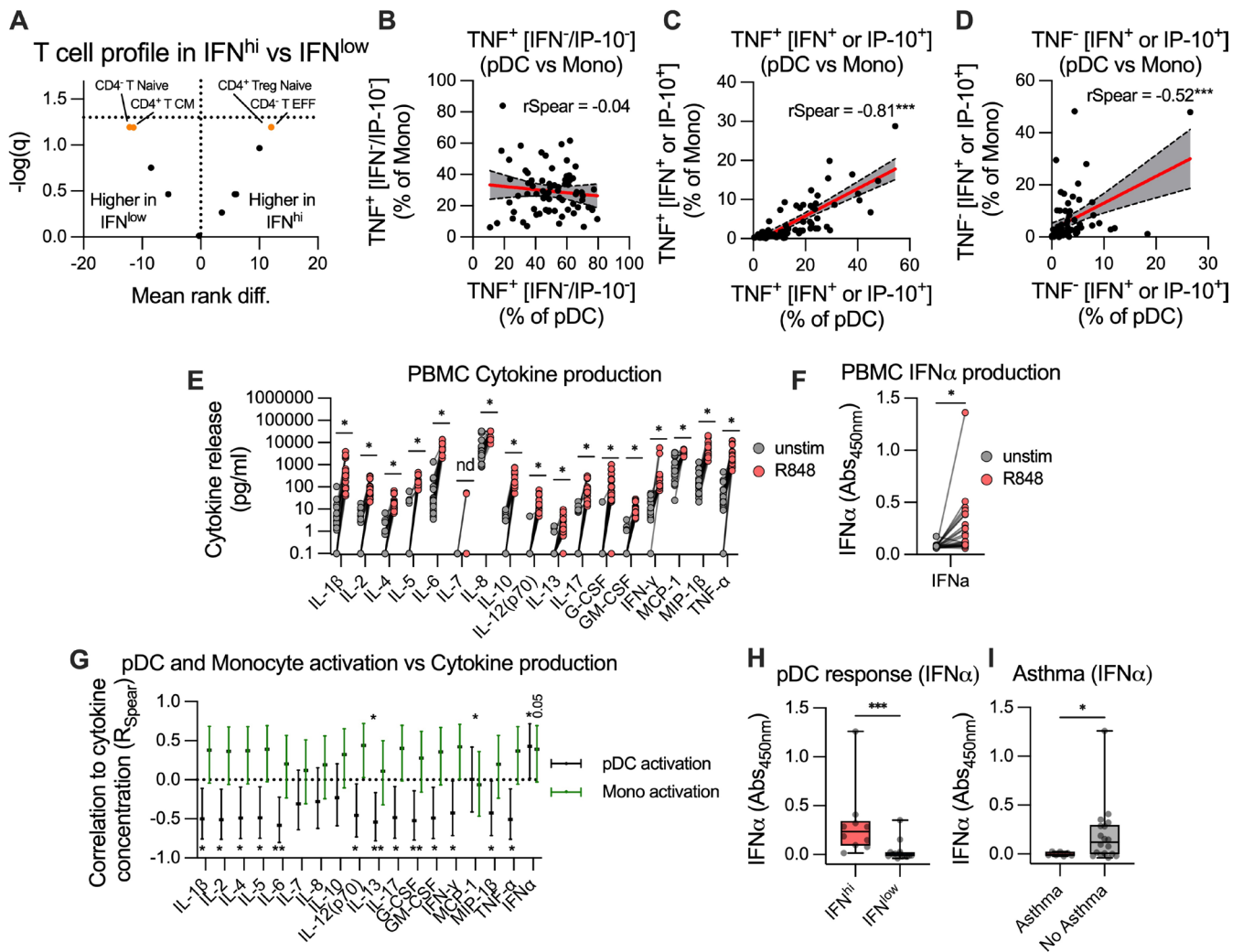
As pDC function is important for shaping T cell responses, we assessed if IFN<sup>hi</sup> or IFN<sup>low</sup> responses were associated with differences in circulating T cell subsets *ex vivo*. We observed a trend toward an increase in CD4<sup>+</sup> Naive Tregs and CD4<sup>+</sup> T effector cells in IFN<sup>hi</sup> individuals, whereas CD4<sup>+</sup> Naive and CD4<sup>+</sup> T CM cells appeared decreased (Figure 4A). Evaluating the *ex vivo* phenotype of pDC (Figure S2) revealed that IFN<sup>low</sup> response individuals displayed an increased proportion of CD5<sup>+</sup> pDC compared to those with an IFN<sup>hi</sup> response (Figure S5A). A small but significantly increased expression of IRF7 in IFN<sup>low</sup> individuals was also observed (Figure S5B).

In parallel to evaluating pDC-specific responses, we also compared TNF and IFN/IP-10 production in CD14<sup>+</sup> monocytes. Although no correlation in TNF<sup>+</sup> [IFN<sup>-</sup>/IP-10<sup>-</sup>] CD14<sup>+</sup> monocytes and pDC was observed (Figure 4B), a strong correlation with TNF<sup>+</sup> and TNF<sup>-</sup> [IFN<sup>+</sup> or IP-10<sup>+</sup>] cells was observed (Figure 4C,D). Cytokine production from PBMC following R848 stimulation was also evaluated in a subset of culture supernatants (Table S2). As expected, R848 increased the production of most cytokines (Figure 4E), including IFN $\alpha$ , which was measured separately (Figure 4F). Interestingly, total pDC activation, as determined by the proportion of cytokine-producing pDC,

was inversely correlated to the release of most cytokines from the PBMC, except for IFN $\alpha$ , where a positive correlation was observed (Figure 4G). Monocyte activation trended mostly toward a positive association with PBMC cytokines, including for IFN $\alpha$  (Figure 4G). No difference in total cytokine production was observed between IFN<sup>hi</sup> versus IFN<sup>low</sup> individuals or between children with or without current asthma (Figure S5C,D), except for IFN $\alpha$  (Figure 4H,I). These findings suggest that although the IFN-I response to R848 is similar in monocytes and pDC, their activation appears to have opposed associations with the PBMC immune response, except for IFN $\alpha$  which is aligned with both the pDC response clusters and current asthma.

### 3.5 | Limited Transcriptional Difference Observed Between pDC Response Clusters

Following *in vitro* stimulation, pDC from a subset of individuals (Table S2) were isolated for transcriptomic analysis (Figure S6). Multidimensional scaling demonstrated a clear separation between unstimulated and R848-stimulated samples (Figure 5A). Weighted gene co-expression network analysis was performed to evaluate systems-based responses to stimulation (Figure 5B). Out of the 9 modules that were identified, five were differentially expressed following R848 stimulation; these included the turquoise, red, brown, green, and blue modules (Figure 5C). Reactome pathway enrichment analysis suggested that these were enriched for genes related to IFN signaling (turquoise) and generation of secondary messenger molecules (blue) (Figure 5D). The red, brown, and green modules were not enriched for a particular gene pathway. Comparing the fold change of eigengene



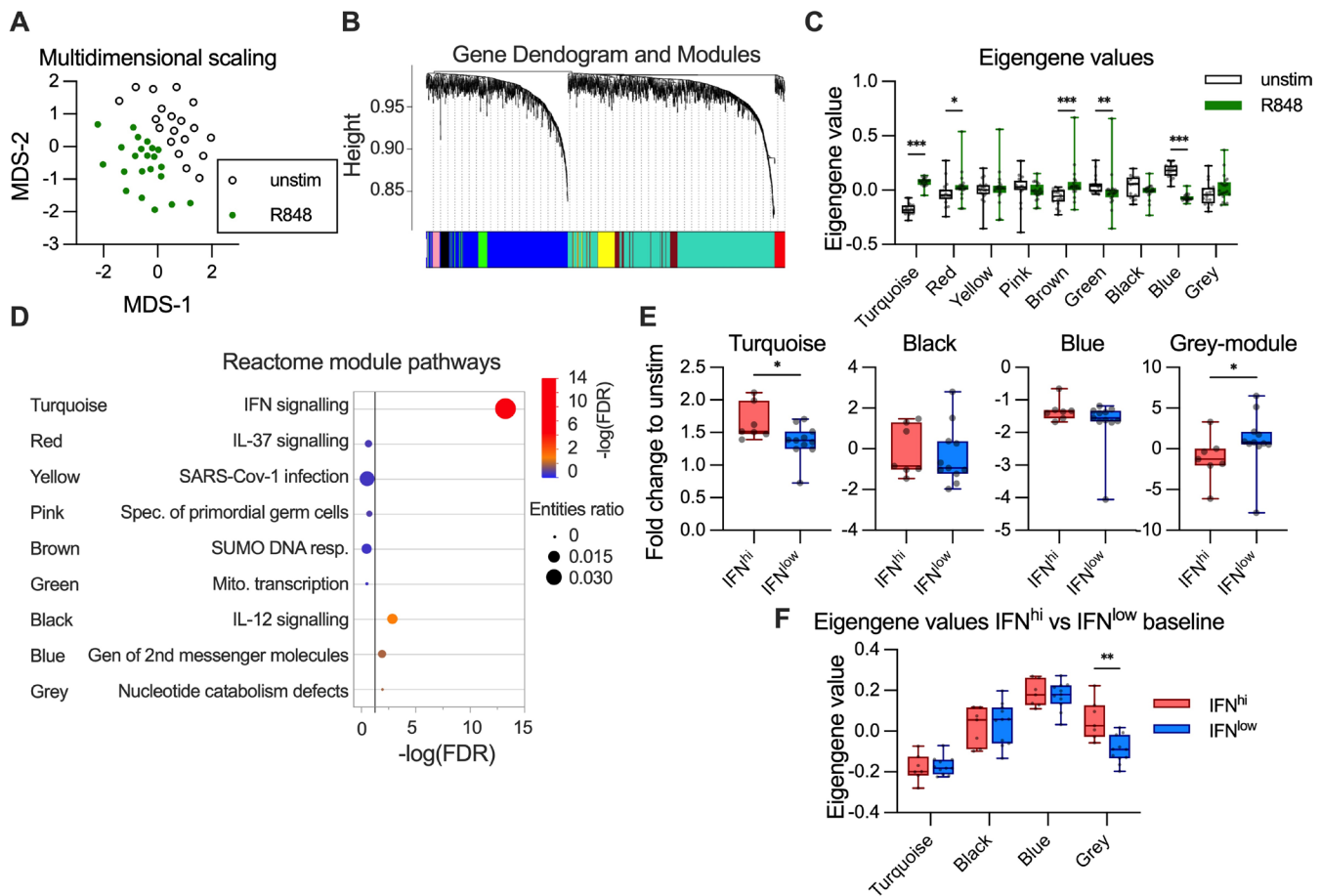
**FIGURE 4** | pDC responses are reflected in pDC and immune cell phenotype. (A) Ratio and significance of immune cell subset abundance in children with an IFN<sup>hi</sup> or IFN<sup>low</sup> response. (B–D) Correlation of TNF<sup>+</sup> [IFN<sup>+</sup>/IP-10<sup>+</sup>] (B), TNF<sup>-</sup> [IFN<sup>+</sup>/IP-10<sup>+</sup>] (C) or TNF<sup>-</sup> [IFN<sup>+</sup> or IP-10<sup>+</sup>] (D) responses in pDC and Monocytes following R848 stimulation. (E, F) Cytokine concentration in culture supernatant in unstimulated (unstim) and R848 stimulated samples from a subset of children. (G) Association with pDC or monocyte activation and cytokine concentration in culture supernatant. (H, I) IFN $\alpha$  production in IFN<sup>hi</sup> and IFN<sup>low</sup> individuals (H) or in children with or without asthma (I) following stimulation. Significance of difference in cell abundance between individuals with an IFN<sup>hi</sup> or IFN<sup>low</sup> pDC or with or without asthma was calculated using the Mann–Whitney test and adjusted for multiple comparisons, if relevant, in (A, H, and I). Spearman’s correlation was used to evaluate the association between cytokine production in pDC and monocytes in (B–D) or between cytokine levels and pDC or monocyte activation in (F). A Wilcoxon paired rank test, following adjustment for multiple comparisons, if relevant, was used to evaluate cytokine responses to R848 in (E, F). *p*-values are displayed as \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

values following R848 stimulation in modules with an associated pathway between IFN<sup>hi</sup> and IFN<sup>low</sup> individuals suggested that the turquoise/IFN module and the grey/nucleotide module responded differentially in individuals with an IFN<sup>hi</sup> versus IFN<sup>low</sup> response (Figure 5E). At baseline, no difference in the turquoise/IFN module was observed, whereas a decreased expression of the grey/nucleotide module was observed in IFN<sup>low</sup> individuals (Figure 5F).

### 3.6 | IgE-Induced pDC Inhibition Is Associated With Reduced Expression of the Blue Module

As IgE binding and IgE crosslinking have been previously shown to modulate production of IFN-I in pDC [11] we

evaluated if IgE crosslinking prior to R848 stimulation impacted the pDC response clusters. As demonstrated by the PBMC cytokine response, IgE crosslinking increased the release of IL-1 $\beta$ , 2, 4, 5, 6, 12, 17, G-CSF, GM-CSF, IFN $\gamma$  as well as TNF, but not IFN $\alpha$ , into the culture supernatant (Figure 6A,B). A small but significant decrease in total pDC activation, as defined by the proportion of cytokine-producing pDC, was also observed (Figure 6C). To assess if IgE crosslinking impacted TNF or IFN/IP-10 producing pDC differently, changes in these subsets were compared. This revealed that TNF producing pDC appeared the most impacted, whereas the impact on IFN or IP-10 producing pDC appeared varied (Figure 6D). No difference in IgE-induced decrease in pDC activation was observed based on the IFN<sup>hi</sup> and IFN<sup>low</sup> pDC response clusters (Figure S7A). We next evaluated if the extent



**FIGURE 5** | pDC specific WGCNA modules and response to stimulation. (A) Multidimensional scaling (MDS) plot of unstimulated (unstim) and R848 stimulated samples. (B) Gene clustering based on Topographical Overlap Matrix (TOM)- based dissimilarity to create WGCNA modules. (C) Comparison of eigengene values in unstim and R848 stimulated samples. (D) Overrepresented Reactome pathways in each module. (E, F) Fold change and baseline levels in module eigengene values in annotated modules following R848 stimulation (E) or at baseline (F) in children with an IFN<sup>hi</sup> or IFN<sup>low</sup> pDC response. Significance of difference following stimulation in (C) or between children with an IFN<sup>hi</sup> or IFN<sup>low</sup> pDC response in (E, F) was calculated using Mann-Whitney tests. *p*-values are displayed as \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

of IgE-induced change in TNF or IFN/IP-10 producing pDC was associated with ex vivo expression of pDC markers. This revealed a significant negative association between ex vivo FcεR1 expression and an IgE-induced reduction of IFN/IP-10 producing pDC. A weak association (*p* = 0.05) with IgE-induced reduction of TNF producing pDC was also observed. Finally, a negative association with pDC-bound IgE ex vivo and IgE-induced reduction of IFN/IP-10 producing pDC was observed (Figure 6E, Figure S7B).

To identify transcriptional drivers of IgE-mediated reduction in pDC activation, the WGCNA module eigengenes were compared in a subset of individuals (Table S2). Following IgE crosslinking, expression of the blue/s messenger module was decreased (Figure 6F). Further inspection of the blue module revealed several interconnected hub genes including TPM2, LILRA4, and CLEC4C (CD303) (Figure 6G). LILRA4 and CLEC4C have previously been implicated in regulating IFN-I responses in pDC [30, 31]. Comparing expression of these genes following IgE crosslinking revealed a trend toward decreased expression of LILRA4 (Figure 6H).

## 4 | Discussion

The current study has demonstrated that pDC responses that lack IFN-I production appear linked to atopy and asthma. We further demonstrated IgE may contribute to shaping these responses and thus extend previous reports suggesting IgE may reduce IFN production. The results also highlight an effect of IgE crosslinking on pDC-mediated TNF production, and link with atopic disease.

We and others have previously observed children with asthma are more likely to mount an insufficient IFN response. Findings from our group suggest this phenotype may be present from birth [19] and observable in children with recurrent wheezing prior to asthma diagnosis [20]. Other studies using PBMC have also identified significant variability in IFN responses following stimulation [32]. Although this study focuses specifically on pDC activation, altered abundance of other circulating immune cells was also observed between children with and without asthma. This included a decreased abundance of cDC2, that may be linked to Th2 inflammation [33]. Focusing on pDC function, clustering in vitro responses

into either IFN<sup>hi</sup> or IFN<sup>low</sup> responses, demonstrating that elevated atopy, eczema, and current asthma are all associated with an IFN<sup>low</sup> response with varied levels of TNF production.

The association with asthma remained significant after adjusting for the level of atopy or the use of asthma medication, suggesting an independent association. Evaluating the

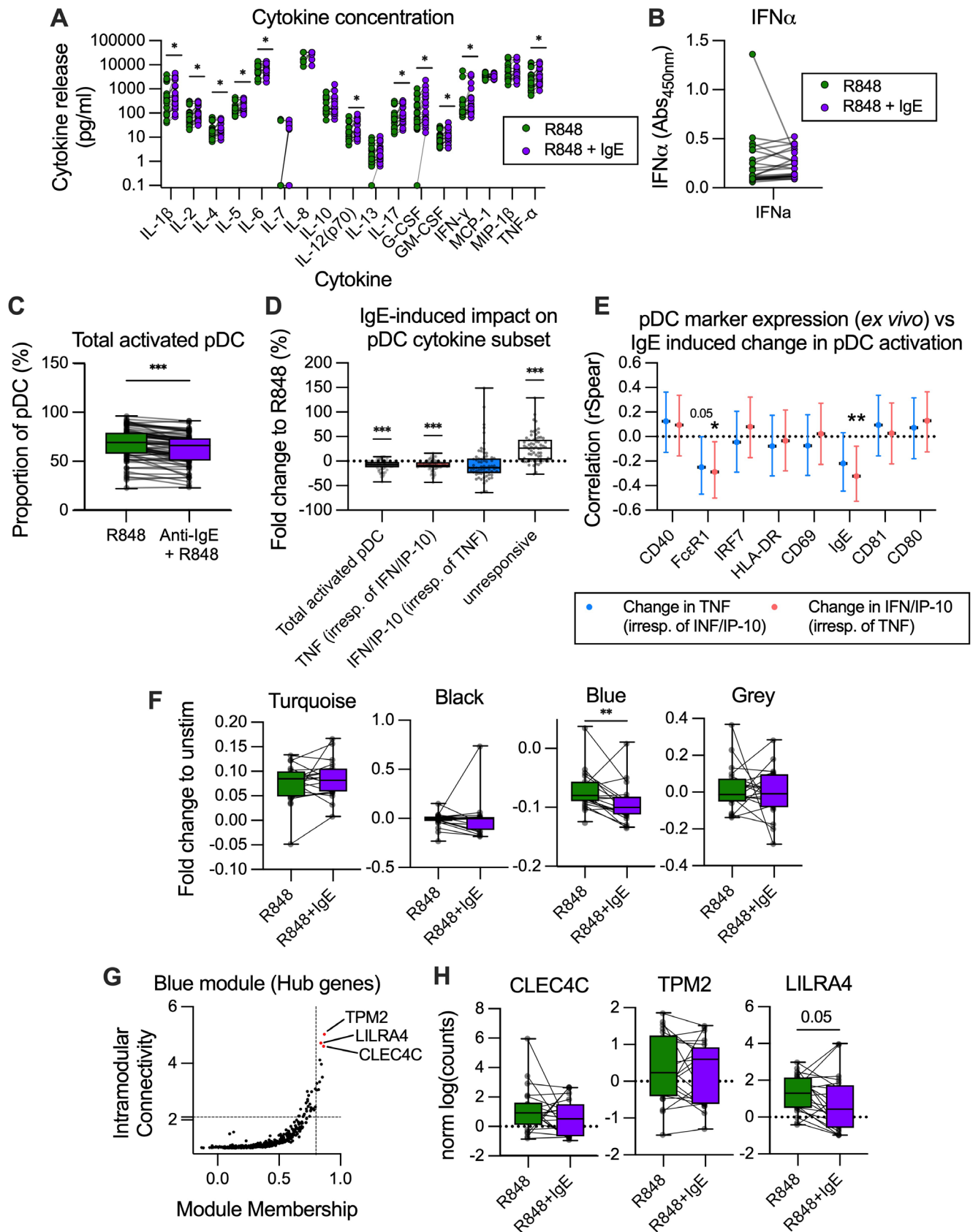


FIGURE 6 | Legend on next page.

**FIGURE 6** | Impact of IgE crosslinking on pDC activation. (A, B) Cytokine concentration in culture supernatant in R848 stimulated samples with or without prior anti-IgE crosslinking. (C) Total pDC activation, based on cytokine production, in R848 stimulated samples with or without prior anti-IgE crosslinking. (D) Change in proportion of specific cytokine producing pDC following anti-IgE crosslinking and subsequent R848 stimulation, displayed as fold change to R848 stimulation only. (E) Association of ex vivo expression of pDC markers and change in proportion of specific cytokine producing pDC following anti-IgE crosslinking. (F) Fold change to unstimulated, in annotated WGCNA modules following R848 stimulation with or without prior anti-IgE crosslinking. (G) Genes within the blue module with high intramodular connectivity and module membership. (H) Difference in gene expression of identified hub genes in R848 stimulated samples with or without prior anti-IgE crosslinking. (A) Wilcoxon paired rank test was used to evaluate cytokine responses in (A, B), significance of difference in pDC activation in (C), and fold change following anti-IgE crosslinking in (D). Spearman's correlation was used to evaluate association between marker expression and IgE-induced change in pDC activation in (E). Differences in module eigengene values in (F) or gene expression levels in (H) were calculated using the Wilcoxon paired rank test. *p*-values are displayed as \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

ex vivo phenotype of pDC suggested the IFN<sup>low</sup> response was associated with an increased abundance of CD5<sup>+</sup> pDC, which aligns with reports suggesting CD5-expressing pDC have reduced IFN-I production [34]. In IFN<sup>hi</sup> individuals, a trend toward increased abundance of CD4<sup>+</sup> Naive Treg was also observed, aligning with demonstrated effects of pDC on CD4<sup>+</sup> Treg induction that also appear sensitive to IgE levels in both atopic [35] and other inflammatory conditions [36]. Counterintuitively, the expression of IRF7, a central gene in IFN-I responses, was increased in IFN<sup>low</sup> individuals. Further investigation into the functional aspect of IRF7 in these individuals may be warranted as the current study did not evaluate the intracellular location or phosphorylation status of IRF7. Increased expression may also be an attempt to restore IFN-I production in IFN<sup>low</sup> individuals. It is important to note that the response phenotypes described in this study were detailed in circulating pDC and limited to stimulation by the TLR7/8 agonist R848. These may differ from IFN-producing ability in the respiratory mucosa [37] of following activation through other receptors, such as RIG-I [38]. Another aspect of our findings is that IFN-I responses in pDC correlated strongly with IFN-I responses in monocytes suggesting a similar regulatory mechanism and was also reflected in released IFN $\alpha$  into the culture supernatant. Little correlation between monocytes and pDC was, however, observed for TNF production suggesting that this is controlled by different mechanisms. It is also interesting to note that pDC activation correlated negatively with PBMC-cytokine production, suggesting a negative influence by pDC, which was reversed by IgE crosslinking. However, IgE-induced basophil activation may also contribute to this observation.

Further studies assessing whether pDC response clusters are either a consequence of developing atopy or already present at birth remain a priority. To address this, the history of early-life virus infections was evaluated without detecting a difference in seasonally adjusted time-to-first infection by RSV or HRV between the two response clusters. We acknowledge that this readout is strongly related to exposures and that other factors, in addition to season, may be of importance that were not captured in this analysis. As part of the ELLF study, weekly nasal swabs were collected, and the presence of viruses was assessed. Counterintuitively, children in the IFN<sup>hi</sup> response cluster displayed a higher frequency of HRV detections than IFN<sup>low</sup> individuals, as well as a trend toward more frequent RSV infections. This appeared driven by a minority of IFN<sup>hi</sup> individuals who had current asthma at 6–7 years of age, potentially suggesting

other vulnerabilities in those individuals. Although there was no difference in mean viral load between the groups, a direct correlation between the proportion of HRV+ tests and LRI-days in IFN<sup>low</sup> individuals was observed. This association was non-existent in IFN<sup>hi</sup> individuals, suggesting that susceptibility to more severe complications with early-life virus infections may differ between the two response groups. IFN<sup>hi</sup> individuals also attended significantly more center-based care compared to IFN<sup>low</sup> individuals; this remained significant after adjusting for asthma. It may be speculated that children attending center-based care are exposed to more pathogens; however, this is likely more important during the first years of life when no difference in attendance was observed. At the age of 7, children spend the majority of their time in school, as opposed to care, making these findings difficult to interpret.

Accompanying the flow-cytometry-based analysis, pDC were also isolated from a subset of participants for transcriptional analysis. R848 stimulation was characterized by upregulation of the turquoise/IFN-dominated gene module. This module was further upregulated in IFN<sup>hi</sup> compared to IFN<sup>low</sup> individuals. At baseline, differences in the grey/small nucleotide catabolism defect module separated IFN<sup>hi</sup> and IFN<sup>low</sup> individuals, although the impact of this remains unclear. Accompanying the cell-specific analysis, evaluation of cytokine production was also performed. Given that no difference in cytokine production, except for IFN $\alpha$  production, was observed across the two pDC response groups, it is unclear what the wider immune implications on an IFN<sup>low</sup> phenotype may be.

The impact of crosslinking cell-bound IgE was also evaluated. Although it is well established that both bound and crosslinked IgE on pDC negatively impact IFN production [10–12] and that treatment with anti-IgE (Omalizumab) may restore IFN-I responses and reduce asthma exacerbations [13–15]. It is less clear how anti-IgE impacts other cytokines produced by pDC or indeed the two response clusters described in this study. Although anti-IgE crosslinking only induced a limited decrease in pDC activation and did not differentially impact the two response clusters, a clear association with the IgE-crosslinking-induced change in TNF and/or IFN/IP-10 production by pDC and baseline Fc $\epsilon$ R1 and pDC-bound IgE was observed, aligning with previous findings. A specific decrease of the blue/s messenger module was also observed, and this module contained several hub genes with relevance for pDC function such as CLEC4C (CD303) and LILRA4 (ILT-7). These genes have both been implicated in the inhibition of IFN $\alpha$  production previously [30, 31],

where LILRA4 is an adaptor protein to FcεR1 and essential for BST2 mediated inhibition of IFN-I production [31, 39]. Further evaluation of baseline protein expression, impact on virus defence, and sensitivity to atopy may provide additional insights into the contribution of these proteins to asthma development.

Finally, it is important to note that the transcriptional analysis was carried out on the total pDC population following stimulation. Inclusion of non-responding cells may have diluted the transcriptional signal from IFN-I producing pDC, as these cells are rare [40] with a median of 12% of total pDC in this study following R848 stimulation. Some effects on IFN-I producing pDC may thus not have been detectable by the bulk sequencing methodology we applied. Another factor separating the flow cytometry and transcriptional analysis was the addition of a Golgi block to samples evaluated by flow cytometry. This was added to allow for detection of intracellular cytokines, but may have limited exposure of pDC to cytokines produced by adjacent cells, including adjacent pDC.

In summary, this study demonstrates that in vitro pDC responses in 7-year-old children are strongly associated with atopy and asthma. However, associations with early-life viral infections are limited, leaving it unclear whether this represents an inherited predisposition. Further evaluation of the development and longitudinal stability of these responses during early life is warranted and may provide biomarkers for the risk of future disease or uncover novel therapeutic targets that can reduce asthma development in children.

#### Author Contributions

**Isabelle Coenen:** methodology, formal analysis, investigation, writing – original draft, writing – review and editing. **Anya C. Jones:** formal analysis, writing – review and editing. **Alice A. White:** methodology, investigation, writing – review and editing. **Mari Takashima:** resources, data curation, writing – review and editing. **Wen Ray Lee:** resources, data curation, writing – review and editing. **Matthew D. Wong:** resources, data curation, writing – review and editing. **Dwan Vilcins:** resources, data curation, writing – review and editing. **Ulrich Kadolsky:** data curation, writing – review and editing. **Ali Sadiq Cheema:** investigation. **Alka Saxena:** writing – review and editing, supervision. **Anthony Bosco:** writing – review and editing, supervision. **Keith Grimwood:** resources, data curation, writing – review and editing. **Peter D. Sly:** conceptualization, resources, writing – review and editing, supervision. **Deborah H. Strickland:** conceptualization, writing – review and editing. **Jonatan Leffler:** conceptualization, methodology, formal analysis, writing – original draft, writing – review and editing, visualization, supervision, project administration, funding acquisition.

#### Acknowledgements

The authors would like to acknowledge all study participants, their families, and the study recruitment team. The authors would also like to acknowledge assistance with cell sorting from Pradeep Kumar and Stephen Proksch at The Kids Research Institute, the bioinformatics team at Genomics WA for their assistance with the processing and QC analysis of the RNA-seq data. The study was funded by the Wal-yan Respiratory Research Centre, and J.L. is supported by a fellowship from the Stan Perron Charitable Foundation. Open access publishing facilitated by The University of Western Australia, as part of the Wiley - The University of Western Australia agreement via the Council of Australian University Librarians.

#### Conflicts of Interest

A.B. is a co-founder, equity holder, and director of the startup company Respiradigm Pty Ltd. that is related to this work. A.B. is the founder of INSiGENe Pty Ltd.; the rest of the authors declare no conflicts of interest.

#### Data Availability Statement

The data that support the findings of this study are openly available in GEO at <https://www.ncbi.nlm.nih.gov/geo>, reference number GSE289352.

#### References

1. F. D. Martinez, A. L. Wright, L. M. Taussig, C. J. Holberg, M. Halonen, and W. J. Morgan, “Asthma and Wheezing in the First Six Years of Life. The Group Health Medical Associates,” *New England Journal of Medicine* 332, no. 3 (1995): 133–138, <https://doi.org/10.1056/NEJM199501193320301>.
2. J. E. Gern, “Immune Responses to Rhinoviruses and Asthma: Are We Three Steps Closer to the Door?,” *Journal of Allergy and Clinical Immunology* 146 (2020): 513–514, <https://doi.org/10.1016/j.jaci.2020.06.031>.
3. P. G. Holt and P. D. Sly, “Viral Infections and Atopy in Asthma Pathogenesis: New Rationales for Asthma Prevention and Treatment,” *Nature Medicine* 18, no. 5 (2012): 726–735, <https://doi.org/10.1038/nm.2768>.
4. T. Jartti and J. E. Gern, “Role of Viral Infections in the Development and Exacerbation of Asthma in Children,” *Journal of Allergy and Clinical Immunology* 140, no. 4 (2017): 895–906, <https://doi.org/10.1016/j.jaci.2017.08.003>.
5. M. M. Kusel, T. Kebabdz, S. L. Johnston, P. G. Holt, and P. D. Sly, “Febrile Respiratory Illnesses in Infancy and Atopy Are Risk Factors for Persistent Asthma and Wheeze,” *European Respiratory Journal* 39, no. 4 (2012): 876–882, <https://doi.org/10.1183/09031936.00193310>.
6. J. F. Read and A. Bosco, “Decoding Susceptibility to Respiratory Viral Infections and Asthma Inception in Children,” *International Journal of Molecular Sciences* 21, no. 17 (2020): 6372, <https://doi.org/10.3390/ijms21176372>.
7. H. Makrinioti, A. Bush, J. Gern, et al., “The Role of Interferons in Driving Susceptibility to Asthma Following Bronchiolitis: Controversies and Research Gaps,” *Frontiers in Immunology* 12 (2021): 761660, <https://doi.org/10.3389/fimmu.2021.761660>.
8. Y. Xi and J. W. Upham, “Plasmacytoid Dendritic Cells and Asthma: A Review of Current Knowledge,” *Expert Review of Respiratory Medicine* 14, no. 11 (2020): 1095–1106, <https://doi.org/10.1080/17476348.2020.1803741>.
9. Y. Xi, N. M. Troy, D. Anderson, et al., “Critical Role of Plasmacytoid Dendritic Cells in Regulating Gene Expression and Innate Immune Responses to Human Rhinovirus-16,” *Frontiers in Immunology* 8 (2017): 1351, <https://doi.org/10.3389/fimmu.2017.01351>.
10. J. T. Schroeder, A. P. Bieneman, H. Xiao, et al., “TLR9- and FcεRI-Mediated Responses Oppose One Another in Plasmacytoid Dendritic Cells by Down-Regulating Receptor Expression,” *Journal of Immunology* 175, no. 9 (2005): 5724–5731, <https://doi.org/10.4049/jimmunol.175.9.5724>.
11. M. A. Gill, G. Bajwa, T. A. George, et al., “Counterregulation Between the FcεRI Pathway and Antiviral Responses in Human Plasmacytoid Dendritic Cells,” *Journal of Immunology* 184, no. 11 (2010): 5999–6006, <https://doi.org/10.4049/jimmunol.0901194>.
12. S. R. Durrani, D. J. Montville, A. S. Pratt, et al., “Innate Immune Responses to Rhinovirus Are Reduced by the High-Affinity IgE Receptor in Allergic Asthmatic Children,” *Journal of Allergy and Clinical Immunology* 130, no. 2 (2012): 489–495, <https://doi.org/10.1016/j.jaci.2012.05.023>.

13. M. A. Gill, A. H. Liu, A. Calatroni, et al., "Enhanced Plasmacytoid Dendritic Cell Antiviral Responses After Omalizumab," *Journal of Allergy and Clinical Immunology* 141, no. 5 (2018): 1735–1743.e9, <https://doi.org/10.1016/j.jaci.2017.07.035>.
14. W. W. Busse, W. J. Morgan, P. J. Gergen, et al., "Randomized Trial of Omalizumab (Anti-IgE) for Asthma in Inner-City Children," *New England Journal of Medicine* 364, no. 11 (2011): 1005–1015, <https://doi.org/10.1056/NEJMoa1009705>.
15. S. J. Teach, M. A. Gill, A. Togias, et al., "Preseasonal Treatment With Either Omalizumab or an Inhaled Corticosteroid Boost to Prevent Fall Asthma Exacerbations," *Journal of Allergy and Clinical Immunology* 136, no. 6 (2015): 1476–1485, <https://doi.org/10.1016/j.jaci.2015.09.008>.
16. M. Vasudev, D. S. Cheung, H. Pincsak, et al., "Expression of High-Affinity IgE Receptor on Human Peripheral Blood Dendritic Cells in Children," *PLoS One* 7, no. 2 (2012): e32556, <https://doi.org/10.1371/journal.pone.0032556>.
17. J. Leffler, J. F. Read, A. C. Jones, et al., "Progressive Increase of FcεpsilonRI Expression Across Several PBMC Subsets Is Associated With Atopy and Atopic Asthma Within School-Aged Children," *Pediatric Allergy and Immunology* 30, no. 6 (2019): 646–653, <https://doi.org/10.1111/pai.13063>.
18. J. W. Upham, G. Zhang, A. Rate, et al., "Plasmacytoid Dendritic Cells During Infancy Are Inversely Associated With Childhood Respiratory Tract Infections and Wheezing," *Journal of Allergy and Clinical Immunology* 124, no. 4 (2009): 707–713, <https://doi.org/10.1016/j.jaci.2009.07.009>.
19. P. G. Holt, D. Mok, D. Panda, et al., "Developmental Regulation of Type 1 and Type 3 Interferon Production and Risk for Infant Infections and Asthma Development," *Journal of Allergy and Clinical Immunology* 143, no. 3 (2019): 1176–1182.e5, <https://doi.org/10.1016/j.jaci.2018.08.035>.
20. I. Coenen, E. de Jong, A. C. Jones, et al., "Impaired Interferon Response in Plasmacytoid Dendritic Cells From Children With Persistent Wheeze," *Journal of Allergy and Clinical Immunology* 153, no. 4 (2024): 1083–1094, <https://doi.org/10.1016/j.jaci.2023.11.920>.
21. M. D. Wong, T. L. Blake, S. F. Zahir, et al., "Longitudinal Tracking of Intra-breath Respiratory Impedance in Preschool Children," *Pediatric Pulmonology* 59, no. 7 (2024): 1885–1893, <https://doi.org/10.1002/ppul.26994>.
22. P. D. Sly, S. A. Cormier, S. Lomnicki, J. N. Harding, and K. Grimwood, "Environmentally Persistent Free Radicals: Linking Air Pollution and Poor Respiratory Health?," *American Journal of Respiratory and Critical Care Medicine* 200, no. 8 (2019): 1062–1063, <https://doi.org/10.1164/rccm.201903-0675LE>.
23. S. B. Lambert, R. S. Ware, A. L. Cook, et al., "Observational Research in Childhood Infectious Diseases (ORChID): A Dynamic Birth Cohort Study," *BMJ Open* 2, no. 6 (2012): e002134, <https://doi.org/10.1136/bmjopen-2012-002134>.
24. M. Sarna, R. S. Ware, S. B. Lambert, T. P. Sloots, M. D. Nissen, and K. Grimwood, "Timing of First Respiratory Virus Detections in Infants: A Community-Based Birth Cohort Study," *Journal of Infectious Diseases* 217, no. 3 (2018): 418–427, <https://doi.org/10.1093/infdis/jix599>.
25. M. D. Takashima, K. Grimwood, D. Vilcins, et al., "Association of Antenatal and Early Childhood Air Pollution and Greenspace Exposures With Respiratory Pathogen Upper Airway Acquisitions and Respiratory Health Outcomes," *International Journal of Environmental Health Research* 34, no. 9 (2024): 3147–3160, <https://doi.org/10.1080/09603123.2023.2299225>.
26. M. D. Takashima, K. Grimwood, P. D. Sly, S. B. Lambert, and R. S. Ware, "Association of Rhinovirus and Potentially Pathogenic Bacterial Detections in the First 3 Months of Life With Subsequent Wheezing in Childhood," *Pediatric Pulmonology* 58, no. 12 (2023): 3428–3436, <https://doi.org/10.1002/ppul.26667>.
27. M. D. Takashima, K. Grimwood, P. D. Sly, et al., "Epidemiology of Respiratory Syncytial Virus in a Community Birth Cohort of Infants in the First 2 Years of Life," *European Journal of Pediatrics* 180, no. 7 (2021): 2125–2135, <https://doi.org/10.1007/s00431-021-03998-0>.
28. C. Gamez, J. Leffler, S. Clark, et al., "Egg-Sensitized Infants Have Elevated CD4(+) Effector Memory T Regulatory Cells From Birth," *Clinical and Experimental Allergy* 54, no. 1 (2024): 34–45, <https://doi.org/10.1111/cea.14431>.
29. Y. Benjamini, A. M. Krieger, and D. Yekutieli, "Adaptive Linear Step-Up Procedures That Control the False Discovery Rate," *Biometrika* 93, no. 3 (2006): 491–507.
30. A. Dzionek, Y. Sohma, J. Nagafune, et al., "BDCA-2, a Novel Plasmacytoid Dendritic Cell-Specific Type II C-Type Lectin, Mediates Antigen Capture and Is a Potent Inhibitor of Interferon Alpha/Beta Induction," *Journal of Experimental Medicine* 194, no. 12 (2001): 1823–1834, <https://doi.org/10.1084/jem.194.12.1823>.
31. W. Cao, D. B. Rosen, T. Ito, et al., "Plasmacytoid Dendritic Cell-Specific Receptor ILT7-Fc EpsilonRI Gamma Inhibits Toll-Like Receptor-Induced Interferon Production," *Journal of Experimental Medicine* 203, no. 6 (2006): 1399–1405, <https://doi.org/10.1084/jem.20052454>.
32. L. M. Murray, G. Thillaiyampalam, Y. Xi, A. S. Cristino, and J. W. Upham, "Whole Transcriptome Analysis of High and Low IFN-Alpha Producers Reveals Differential Response Patterns Following Rhinovirus Stimulation," *Clinical & Translational Immunology* 10, no. 11 (2021): e1356, <https://doi.org/10.1002/cti2.1356>.
33. F. Ronchese, G. R. Webb, S. Ochiai, O. Lamiabile, and M. Brewerton, "How Type-2 Dendritic Cells Induce Th2 Differentiation: Instruction, Repression, or Fostering T Cell-T Cell Communication?," *Allergy* 80, no. 2 (2025): 395–407, <https://doi.org/10.1111/all.16337>.
34. H. Zhang, J. D. Gregorio, T. Iwahori, et al., "A Distinct Subset of Plasmacytoid Dendritic Cells Induces Activation and Differentiation of B and T Lymphocytes," *Proceedings of the National Academy of Sciences of the United States of America* 114, no. 8 (2017): 1988–1993, <https://doi.org/10.1073/pnas.1610630114>.
35. J. Lopez-Abente, C. Benito-Villalvilla, X. Jaumont, P. Pfister, P. Tassinari, and O. Palomares, "Omalizumab Restores the Ability of Human Plasmacytoid Dendritic Cells to Induce Foxp3(+)-Tregs," *European Respiratory Journal* 57, no. 1 (2021): 2000751, <https://doi.org/10.1183/13993003.00751-2020>.
36. A. de la Rocha-Munoz, C. Benito-Villalvilla, D. Olivares, et al., "The Role of IgE in Crohn's Disease by Impairing the Capacity of Plasmacytoid Dendritic Cells to Generate FOXP3(+) Tregs," *Allergy* (2025), <https://doi.org/10.1111/all.16517>.
37. S. Vangeti, J. Gertow, M. Yu, et al., "Human Blood and Tonsil Plasmacytoid Dendritic Cells Display Similar Gene Expression Profiles but Exhibit Differential Type I IFN Responses to Influenza A Virus Infection," *Journal of Immunology* 202, no. 7 (2019): 2069–2081, <https://doi.org/10.4049/jimmunol.1801191>.
38. A. Szabo, Z. Magyarics, K. Pazmandi, L. Gopcsa, E. Rajnavolgyi, and A. Bacsi, "TLR Ligands Upregulate RIG-I Expression in Human Plasmacytoid Dendritic Cells in a Type I IFN-Independent Manner," *Immunology and Cell Biology* 92, no. 8 (2014): 671–678, <https://doi.org/10.1038/icb.2014.38>.
39. W. Cao, L. Bover, M. Cho, et al., "Regulation of TLR7/9 Responses in Plasmacytoid Dendritic Cells by BST2 and ILT7 Receptor Interaction," *Journal of Experimental Medicine* 206, no. 7 (2009): 1603–1614, <https://doi.org/10.1084/jem.20090547>.
40. F. Wimmers, N. Subedi, N. van Buuringen, et al., "Single-Cell Analysis Reveals That Stochasticity and Paracrine Signaling Control Interferon-Alpha Production by Plasmacytoid Dendritic Cells," *Nature Communications* 9 (2018): 3317, <https://doi.org/10.1038/s41467-018-05784-3>.

## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1.** Gating strategy for identification of immune cell populations from the general immune cell panel. Data from one representative individual is displayed. **Figure S2.** Gating strategy for identification of immune cell subsets from the pDC/B cell spectral panel. Data from one representative individual is displayed. **Figure S3.** Gating strategy for detection of cytokine production in pDC and Monocytes following stimulation with R848. Data from one representative individual is displayed. **Figure S4.** (A) Association of pDC responses following adjustment for current use of asthma therapies. (B, C) are attendance (y/n) at each age for IFN<sup>hi</sup> and IFN<sup>low</sup> individuals. (C) hours per week at specific type of care locations for IFN<sup>hi</sup> and IFN<sup>low</sup> individuals. (D) Association of attending centre-based care adjusted for current asthma status in IFN<sup>low</sup> individuals. (E) Association of IFN<sup>low</sup> pDC responses with asthma following adjustment for wheal size. Statistical significance of differences in (B, C) was calculated using a Mann–Whitney test adjusted for multiple comparisons. *p*-values are displayed as \*\**p* < 0.01, \**p* < 0.05 or as indicated. **Figure S5.** (A, B) Distribution of ex vivo pDC subsets (A) or expression of markers associated with pDC function (B) in individuals with an IFN<sup>hi</sup> or IFN<sup>low</sup> pDC response. (C, D) Comparison of cytokine concentration in culture supernatant in children with an IFN<sup>hi</sup> versus IFN<sup>low</sup> (C) or with or without current asthma (D). Statistical significance of differences in (A–D) was calculated using a Mann–Whitney test adjusted for multiple comparisons. *p*-values are displayed as \**p* < 0.05. **Figure S6.** Gating strategy for flow cytometry assisted cell sorting of pDC following culture. Data from one representative individual is displayed. **Figure S7.** (A) change in total pDC activation between IFN<sup>hi</sup> and IFN<sup>low</sup> response clusters. (B) Correlation plots for change in TNF and IFN/IP-10 producing pDC following IgE crosslinking and FcεR1 and IgE expression. Statistical significance of differences in A was calculated using a Mann–Whitney test and associations were evaluated using Spearman's correlation. *p*-values are displayed as \**p* < 0.05 or as indicated.