Respiratory infections regulated blood cells IFN-β-PD-L1 pathway in pediatric asthma

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Abstract

Background: Respiratory infections, in general, and rhinovirus infection specifically are the main reason for asthma exacerbation in children and programmed cell death protein 1 ligand (PD-L1) expression inhibits T cell responses. Objective: Could the interferon (IFN) type I expression in peripheral blood mononuclear cells (PBMCs) improve disease exacerbation in pediatric asthma? Results: Here we found increased level of PD-L1 messenger RNA (mRNA) in total blood cells isolated from preschool children with virus-induced asthma, with lower percentage of forced expiratory volume in 1 second and with high serum levels of the C-reactive-protein.

Conclusions and Clinical Relevance: These data indicate that, in the presence of infection in the airways of preschool children, worse asthma is associated with induced PD-L1 mRNA expression in blood cells. Further, type I IFN, IFN-β, a cytokine that is involved in the clearance of infections, was found to be associated with a better lung function in asthmatic children. These data suggest that improving peripheral blood IFN type I expression in PBMCs in pediatric asthma could improve disease exacerbation due to suppressing PD-L1 expression in blood cells.

KEYWORDS
human rhinovirus, IFNβ, PD-L1, pediatric asthma

1 INTRODUCTION

The immune responses of the host to respiratory infections, in general, and to rhinovirus (RV) infection in particular, are associated with upregulation of type I interferon (IFN) pathways1,2 in the airways and systemically in the blood cells.3 Deficient systemic IFN responses to respiratory infections have been observed in patients with noncontrolled asthma,1,2,4,5 suggesting that type I IFN could be used to improve lung function in asthma. IFN response of the host can be suppressed by infectious agents by upregulation of programmed cell death protein 1 ligand (PD-L1),2,6 which then inhibit T cell proliferation via binding to programmed cell death protein 1 (PD1), considered as an immune checkpoint because it downregulates the immune responses.2

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To analyze the influence of RV on IFN responses in asthma, we concentrated on the influence of human RV in the airways on IFN-induced PD-L1 in the peripheral blood cells of children with and without asthma.⁸,⁹

2 | METHODS

2.1 | Human study PreDicta

In the European Study PreDicta (post-infectious immune reprogramming and its association with persistence and chronicity of respiratory allergic diseases), we examined healthy and asthmatic preschool children at the age of 4 to 6 years in collaboration with the children hospital in Erlangen. The study in Erlangen was approved by the ethics committee of the Friedrich-Alexander University Erlangen-Nürnberg, Germany (Re-No 4435) and it is registered in the German Clinical Trials Register (www.germanctr.de; DRKS00004914).

Two cohorts of preschool children (age 4-6 years) with and without asthma were analyzed. The recruitment of the subjects, inclusion and exclusion criteria as well as the timescale for clinical visits and data collection were exactly described recently¹,²,⁵,⁶ along with the clinical aspects and characteristics and reported in other form in Tables 1 and 2.

For gene expression analysis, we isolated messenger RNA (mRNA) from total blood cells of the children as previously described and performed quantitative real-time polymerase chain reaction (PCR) as described below.¹ The levels of C-reactive-protein (CRP) in the serum samples of the children were measured by turbidimetry on a Roche

| Patient | Skin prick test⁴ | Atopic dermatitis | Microbial swab result | FEV1% predicted¹ | PEF% predicted¹ | CRP, mg/L |
|---------|------------------|------------------|-----------------------|------------------|----------------|-----------|
| 208     | n.d.             | No               | RV−                   | 77               | 75             | n.d.      |
| 211     | n.d.             | No               | RV+                   | 121              | 94             | 1.40      |
| 214     | n.d.             | No               | RV+                   | 110              | 94             | 0.29      |
| 215     | n.d.             | No               | RV−                   | 118              | 78             | 0.90      |
| 218     | n.d.             | No               | RV+                   | 111              | 92             | 0.68      |
| 219     | n.d.             | No               | RV+                   | 107              | n.d.           | 0.48      |
| 220     | negative         | No               | RV−                   | 84               | 60             | 0.78      |
| 221     | n.d.             | No               | RV+                   | n.d.             | n.d.           | 0.26      |
| 222     | n.d.             | Yes              | RV−                   | 105              | 86             | 0.22      |
| 226     | n.d.             | No               | RV+                   | 109              | 93             | 1.25      |
| 227     | n.d.             | No               | RV+                   | 87               | 95             | 21.92     |
| 232     | negative         | No               | RV+                   | 100              | 70             | 0.76      |
| 233     | n.d.             | No               | RV+                   | 112              | 105            | 0.79      |
| 234     | al               | No               | RV+                   | 119              | 95             | 1.74      |
| 235     | ca, f            | No               | RV+                   | 113              | 75             | n.d.      |
| 236     | n.d.             | No               | RV−                   | 111              | 101            | 0.11      |
| 237     | negative         | No               | RV−                   | 109              | 101            | 2.16      |
| 240     | negative         | No               | RV+                   | 92               | 74             | 0.64      |
| 241     | negative         | No               | RV+                   | 123              | 79             | 0.36      |
| 245     | negative         | No               | RV−                   | 121              | 106            | 0.51      |
| 246     | negative         | Yes              | RV+                   | 109              | 92             | 0.74      |
| Average | Pos. = 9.5%      | Yes = 9.5%       | RV + = 66.7%          | 106.9 ± 2.9      | 87.6 ± 3.0     | 1.89 ± 1.12 |
|         | Neg. = 33.3%     | No = 90.5%       | RV− = 33.3%           |                  |                |           |

Abbreviations: CRP, C-reactive-protein; FEV1, forced expiratory volume in 1s/forced vital capacity; PEF, peak expiratory flow; RV, rhinovirus.

⁴al, Alternaria species; ca, cat; f, Dermatophagoides mix; n.d., not done.

⁵Lung function results pre-bronchodilation.
| Patient | Asthma severity | Phenotype | Skin prick test | Treatment | Atopic dermatitis | Microbial swab result | FEV1% predicted | PEF% predicted | CRP, mg/L |
|---------|----------------|-----------|----------------|-----------|------------------|-----------------------|-----------------|----------------|------------|
| 201     | I              | v         | al, ca, g      | Steroid   | Yes              | RV+                   | 126             | 132            | 0.4        |
| 202     | II             | u         | al, b, g       | Steroid   | Yes              | RV+                   | 111             | -              | 0.31       |
| 203     | II             | u         | ca             | Steroid   | No               | RV−                   | 95              | 80             | 2.12       |
| 204     | II             | a         | al, am, ca, f, g | Steroid  | Yes              | RV−                   | 128             | 127            | 0.31       |
| 205     | I              | u         | ca             | Steroid   | No               | RV−                   | 102             | 86             | 2.13       |
| 206     | I              | u         | al             | Steroid   | No               | RV+                   | 129             | 119            | 0.69       |
| 207     | I              | v         | g              | Steroid   | Yes              | RV−                   | 143             | 117            | 0.13       |
| 209     | II             | v, a      | g              | Steroid   | Yes              | RV−                   | 115             | 88             | /          |
| 210     | I              | v         | b, g           | Nonsteroid| Yes              | RV−                   | 98              | 77             | 5.34       |
| 212     | II             | e         | v              | Steroid   | No               | RV−                   | 96              | 84             | /          |
| 213     | III            | e         | negative       | Steroid   | No               | RV+                   | 115             | 106            | 0.13       |
| 216     | III            | a, v      | ca, f, g       | Steroid   | No               | RV−                   | 92              | 75             | 0.50       |
| 217     | I              | a, e, v   | b, ca, f, g    | Steroid   | Yes              | RV−                   | 111             | 104            | 1.01       |
| 223     | I              | v         | ca, f, g       | Steroid   | Yes              | RV+                   | 99              | 90             | 0.63       |
| 224     | I              | v         | negative       | Steroid   | No               | RV+                   | 135             | 107            | 0.39       |
| 225     | I              | v         | negative       | Steroid   | No               | RV+                   | 99              | 82             | /          |
| 228     | I              | v         | ca, f, g       | Nonsteroid| No               | RV−                   | 88              | 65             | 0.37       |
| 229     | I              | v         | al, b, ca, f, g | Nonsteroid| Yes              | RV+                   | 87              | 65             | /          |
| 230     | I              | v         | al, am, b, ca, f, g | Nonsteroid| Yes              | RV+                   | 101             | 86             | 0.69       |
| 231     | I              | v         | b              | Steroid   | No               | RV−                   | 71              | 60             | 1.87       |
| 238     | I              | v         | negative       | Steroid   | No               | RV+                   | 77              | 54             | 20.33      |
| 239     | I              | e         | n.d.           | Nonsteroid| No               | RV+                   | 98              | 92             | 0.55       |
| 242     | II             | a, e, v   | al, b, ca, f, g | Steroid  | No               | RV+                   | 81              | 99             | 0.64       |
| 243     | II             | v         | negative       | Steroid   | No               | RV+                   | 69              | 53             | 2.90       |
Integra 800 Analyzer (CRPL2 reagent, limit of detection 1.0 mg/L, interday CV 1.4% [8.1 mg/L]; Roche Diagnostics, Basel, Switzerland). The detection of RV in nasopharyngeal swab obtained from the children was performed at the Department of Virology, University of Turku (Finland). The description of this procedure is already published in detail elsewhere.\(^1\)

### 2.2 FEV1 and PEF

The percentage of forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and peak expiratory flow (PEF) were measured at baseline visit (B0) by using spirometry. After a period of normal breathing, the participant should inhale maximal, directly followed by maximal and fast exhalation. The volume exhaled in 1 second is FEV1. The total exhaled volume is FVC. The ratio FEV1/FVC is stated as FEV1%. The PEF is defined as the largest expiratory flow, which is achieved with a maximum forced effort after maximum inspiration.

### 2.3 Human RNA isolation from Tempus Tubes and quantitative real-time polymerase chain reaction

At baseline visit, whole blood was collected in Tempus® Blood RNA Tubes (Life Technologies™, GmbH, Darmstadt, Germany) and RNA was extracted with the MagMax for Stabilized Blood Tubes RNA Isolation Kit. For reverse transcription of RNA (1 µg), we used the first strand complementary DNA (cDNA) synthesis kit for RT-PCR (MBI Fermentas, St. Leon-Rot, Germany). The resulting template cDNA was then amplified by quantitative real-time PCR (qPCR) using SoFast EvaGreen Supermix (Bio-Rad Laboratories, München, Germany). The qPCR itself was performed in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories) with a cycle of 2 minutes at 98°C, 50 cycles of 5 seconds at 95°C, 10 seconds at 60°C, followed by 5 seconds at 65°C and 5 seconds at 95°C. The primer sequences used for the real-time PCR are listed in Table S1. The mRNA of the genes of interest was normalized using the housekeeping gene hypoxanthine guanine phosphoribosyl transferase (HPRT).

### 2.4 Isolation of peripheral blood mononuclear cells, in vitro cell culture, and analysis of the cell supernatants

At the time of recruitment (baseline visit), peripheral blood mononuclear cells (PBMCs) were isolated from
heparinized blood with Ficoll using density centrifugation. After isolation, PBMC numbers were adjusted to a concentration of 10⁶ viable cells/mL in complete culture medium. For cell culture, Roswell Park Memorial Institute 1640 medium supplemented with 25 mmol/L HEPES (Gibco, Invitrogen, Darmstadt, Germany) was used. Furthermore, 100 IU/mL penicillin, 100 µg/mL streptomycin, 50 µmol/L β-mercaptoethanol, 1% L-glutamine (200 mmol/L), 1% MEM Vitamin, 1% nonessential amino acids, 1% sodium pyruvate, and 10% fetal bovine serum were added (complete culture medium); these reagents were purchased from Sigma-Aldrich (Steinheim, Germany). The PBMCs were cultured in complete culture medium for 24 hours at 37°C and 5% CO₂, whereby parts of them were challenged in vitro with 10 µg/mL PHA (Sigma-Aldrich) or with RV (RV1b). The growth of RV1b and the description of the RV1b infection itself have been published previously in detail elsewhere.

Human IFNβ and interleukin 10 (IL-10) was detected in the cell-culture supernatants by using IFNβ ELISA kit from PeproTech (Hamburg, Germany) and IL-10 OptEIA™ sandwich ELISA kit from BD Bioscience (Heidelberg, Germany), respectively, according to the manufacturer’s protocol.

2.5 | Statistical analysis

Statistical analysis was performed using Prism (version 7) for Windows (GraphPad, La Jolla, CA). Differences were evaluated for significance by using the two-tailed Student t test or ordinary one-way analysis of variance to generate P-value data (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001) for all data. Unless otherwise indicated, data are presented as mean ± SEM.

3 | RESULTS

3.1 | PD-L1 is induced in blood cells of preschool asthmatic children with a virus-induced asthma phenotype and associated with the presence of rhinovirus in their airways

We recently described that acute in vitro infection of PBMCs from preschool children with and without asthma with RV, a single-stranded RNA picornavirus, is associated with the upregulation of IFN-regulated genes like STAT1, STAT2, and IFN regulatory factor 1.² Moreover, paradoxically, IFNγ upregulates also PD-L1, a factor involved in silencing/exhausting of activated T cells by ligating PD1 on the surface of T cells.¹⁰ Consistently, we found that acute RV infection ex vivo induced PD-L1 and CTLA4 in the PBMCs of asthmatic children.⁶ We thus wanted to follow up these in vitro observations in the two cohorts of our study and analyzed 21 control children and 24 children with asthma (Figure 1A). The clinical data of these cohorts of children were recently reported²,⁵,⁶ and are summarized in Table 1 and 2. By looking at the PD-L1 mRNA expression was induced in children with a virus-induced asthma phenotype (in accordance to PRACTALL guidelines 2008¹¹) compared to healthy control children (Figure 1B). Children with this asthma phenotype shows symptom-free periods, whereas the most common precipitating factor are colds by respiratory viruses, like human RV.¹¹

Furthermore, by trend, we observed an induction of PD-L1 mRNA in the blood cells of asthmatic children as compared to control children (Figure 1C). We next analyzed PD-L1 expression after allergen and RV challenge. Considering the presence of RV (+RV) in the airways, we found that, by trend, asthmatic children with RV in the airways, have an increased PD-L1 mRNA expression in total blood cells (Figure S1a). This is also associated with increased expression of the low-density lipoprotein receptor (Figure S1b), which is one of the main receptors used by the viruses, especially for RV1b, to entering the cells.

3.2 | PD-L1 is upregulated in blood cells of asthmatic children with increased bronchoconstriction

We then asked if the lung function, especially the FEV1% as well as the PEF% (predicted), of the cohorts would correlate with increased PD-L1 expression in blood. The FEV1/FVC ratio (FEV1%) is a calculated ratio used in the diagnosis of obstructive and restrictive lung disease. It represents the proportion of a person’s vital capacity that they are able to expire in the first second of forced expiration (FEV1) to the full, FVC. The result of this ratio is expressed as FEV1%.¹² Lower values of FEV1% represent airway obstruction. In our cohort of children with asthma, but not in control children, we found a PD-L1 induction in children with higher bronchoconstriction (Figure 1D) and an inverse correlation between PD-L1 and FEV1% (Figure 1E), indicating that worse asthma is associated with induction of PD-L1 mRNA in blood cells of children with asthma. We then further investigated the role of another lung function parameter, the PEF% value (Figure 1F,G). The PEF% is defined as the largest expiratory flow, which is achieved with a maximum forced effort after maximum inspiration and is used as a control parameter during asthma therapy. Similar to the FEV1%, we found a significant PD-L1
induction in children with worse asthma (Figure 1F) as well as an inverse correlation between PD-L1 expression and the PEF% (Figure 1G). We also found that increased PD-L1 mRNA expression correlated with reduced FEV1% and PEF% (Figure 1H), indicating that asthmatic preschool children with RV colonization in the airways have worse respiratory function associated with PD-L1 induction in their PBMCs. By contrast, healthy control children with and without RV in the airways as well as in asthmatic children without RV colonization in the airways no correlation between FEV1% or PEF% and PD-L1 was observed (Figure S1c,d).
3.3 | Interferon-β correlated with better lung function in asthmatic children

We next reasoned that in the case of asthma induced by infections, especially RV infections, IFN-β and specifically IFNβ might be of importance.14 Thus, we next analyzed the IFNβ level in cell culture supernatants of untreated PBMCs from healthy and asthmatic children with and without RV in the airways (Figure 2A) as well as after a restimulation with RV1b in vitro (Figure S2a,b) and correlated them with their FEV1% and PEF% (Figure 2A,B; Figure S2c-e and S3). Here we found that, only asthmatic children and especially asthmatic children with RV in the upper airways show a direct correlation between the IFNβ level and the FEV1% and PEF%, respectively, indicating that a subpopulation of children could respond to RV infection with IFNβ production.

3.4 | PD-L1 levels correlated with IFNβ production in healthy but not in asthmatic children

Since it is known that IFN induces PD-L1,14 we correlated the IFNβ expression in the supernatants of untreated and with RV1b restimulated PBMCs and the PD-L1 expression in total blood cells and found a direct correlation in control children, but not in asthmatic children (Figure 3 and Figure S4a,b). These data indicate that IFNβ is associated with PD-L1 in control children and that asthmatic children have a disturbed IFNβ-mediated PD-L1 induction.

3.5 | PD-L1 is upregulated in blood cells of asthmatic children with high C-reactive protein serum levels and correlated with RV in the airways

We next reasoned that not only RV but also other infection or inflammatory agents could cause PD-L1 induction in asthmatic children. We thus next looked at the CRP level in serum of our cohorts of children. CRP binds to the phosphocholine expressed on the surface of dead or dying cells and some bacteria and leading to the activation of the complement system and promotion of phagocytosis by macrophages.15 Higher levels are found in inflammation, viral infections (10-40 mg/L), active bacterial infection (40-200 mg/L), severe bacterial infections, and burns (>200 mg/L).16 We considered high CRP levels as an indicator of ongoing infection and inflammation and found that children with asthma and a CRP value over 5 mg/L had a significantly higher PD-L1 mRNA expression in total blood cells as compared to the control children (Figure 4A). Moreover, in both healthy and asthmatic children, CRP was found to be associated with high PD-L1 levels in the serum (Figure 4B and Figure S4c). Finally, in the presence of RV in the airways, CRP correlated with PD-L1 expression in healthy children (Figure 4C). Taken together, these data suggest the presence of induced PD-L1+ cells in the blood of asthmatics with worse asthma and ongoing inflammation and infection.

4 | DISCUSSION

Here we found increased PD-L1 mRNA levels in total blood cells isolated from preschool asthmatic children with a virus-induced asthma phenotype, lower FEV1% and with high CRP serum levels, indicating that worse asthma, in the presence of infections in the airways, is associated with induced PD-L1 mRNA expression. IFNβ, released by PBMCs in preschool children with HRV infected airways was found to correlate with improved lung function, both in control and asthmatic children. However, although in control children IFNβ directly correlated with PD-L1 mRNA expression, in asthmatic children this correlation was lost in peripheral blood.

PD-L1 has been associated with hepatitis B infections.17 In this case, the use of anti-PD-L1 inhibitors was suggested to improve natural killer T cell function.
FIGURE 2  Interferon-β (IFNβ) correlated with better lung function in asthmatic children. A,B, Correlation of the IFNβ level, measured in the supernatants of the untreated peripheral blood mononuclear cell (PBMC) culture and the respective FEV1% (i) and PEF% (j) of asthmatic children with and without RV in their airways. *P ≤ .05; **P ≤ .01, ***P ≤ .001, ****P ≤ .0001. FEV1, forced expiratory volume in 1 second; PEF, peak expiratory flow; RV, rhinovirus

FIGURE 3  IFNβ correlated with PD-L1 mRNA level in control children but not in asthmatic children. Correlation of the PD-L1/HPRT mRNA level in total blood cells with the IFNβ level, measured in the supernatants of the respective untreated and with RV1b restimulated PBMC culture, of healthy and asthmatic children. *P ≤ .05, **P ≤ .01, ***P ≤ .001, ****P ≤ .0001. IFNβ, interferon-β; mRNA, messenger RNA; PBMC, peripheral blood mononuclear cell; PD-L1, programmed cell death protein 1 ligand
resulting in inhibition of virus replication. This mechanism seems to be similar to a described mechanism in lung cancer where anti-PD-L1 antibody treatment results in ameliorated antitumour immune response. Here we found that PD-L1 mRNA was induced in association with higher levels of the infection marker CRP in the periphery but not with RV in the airways. In addition, PD-L1 mRNA did not directly correlated with IFN-β release in the peripheral blood of asthmatic children with and without RV in their airways. Further, we recently reported that these asthmatic children have prevalent Gram-negative colonization in the airways which are associated with induction of IFN-β release in the airways in their nasal pharyngeal fluid. Thus it is possible that the direct correlation found between CRP and PD-L1 relate to the presence of Gram-negative bacteria in the airways of these children.

Taken together, these data reveal that the host respond to infection with release of IFN-β in blood cells. The infectious agent then redirects this response by up-regulating PD-L1, which inhibits the immune system. In asthma, there seems to be a therapeutical possibility to use IFN type 1 to improve lung function without inducing PD-L1, thus keeping activated anti-infection immune responses.

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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.
AUTHOR CONTRIBUTIONS
JK is the major investigator of this study and PH contributed to the design of this study, supervised this work, and wrote the manuscript. TV did the respiratory virus analysis in the nasal pharyngeal fluid of the children analyzed in this study. AK and TZ are the pediatricians that saw most of the children in Predicta WP1-UKER and made the medical diagnosis. MR did the CRP analysis. NP designed the WP1 project Predicta and was the coordinator of Predicta.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES
1. Bergauer A, Sopel N, Kroß B, et al. IFN-alpha/IFN-lambda responses to respiratory viruses in paediatric asthma. Eur Respir J. 2017a;49(2):1600969. https://doi.org/10.1183/13993003.00969-2016
2. Bergauer A, Sopel N, Kroß B, et al. Rhinovirus species/genotypes and interferon-lambda: subtypes, receptor and polymorphisms—missing pieces of the puzzle of childhood asthma? Eur Respir J. 2017b;49(3):1700265. https://doi.org/10.1183/13993003.00265-2017
3. Hansel TT, Tunstall T, Trujillo-Torralbo MB, et al. A comprehensive evaluation of nasal and bronchial cytokines and chemokines following experimental rhinovirus infection in allergic asthma: increased interferons (IFN-gamma and IFN-lambda) and type 2 inflammation (IL-5 and IL-13). EbioMedicine. 2017;19:128-138. https://doi.org/10.1016/j.ebiom.2017.03.033
4. Sykes A, Macintyre J, Edwards MR, et al. Rhinovirus-induced interferon production is not deficient in well controlled asthma. Thorax. 2014;69(3):240-246. https://doi.org/10.1136/thoraxjnl-2012-202909
5. Hentschke I, Graser A, Melichar VO, et al. IL-33/ST2 immune responses to respiratory bacteria in pediatric asthma. Sci Rep. 2017;7:43426. https://doi.org/10.1038/srep43426
6. Bielor C, Sopel N, Maier A, et al. Role of TGF-beta in anti-rhinovirus immune responses in asthmatic patients. J Allergy Clin Immunol. 2017;140(1):e210. https://doi.org/10.1016/j.jaci.2016.10.049
7. Ruibal P, Oestreicher L, Lüdtke A, et al. Unique human immune signature of Ebola virus disease in Guinea. Nature. 2016;533(7601):100-104. https://doi.org/10.1038/nature17949
8. Liu J, Zhou Y, Yu Q, et al. Higher frequency of CD4+CXCR5+ICOS+PD1+ T follicular helper cells in patients with infectious mononucleosis. Medicine. 2015;94(45):e2061. https://doi.org/10.1097/md.0000000000002061
9. Zhang G, Li N, Zhang P, et al. PD-1 mRNA expression is associated with clinical and viral profile and PD1 3′-untranslated region polymorphism in patients with chronic HBV infection. Immunol Lett. 2014;162(1 Pt A):212-216. https://doi.org/10.1016/j.imlet.2014.09.001
10. Mandai M, Hamanishi J, Abe K, Matsumura N, Baba T, Konishi I. Dual faces of IFN gamma in cancer progression: a role of PD-L1 induction in the determination of pro- and antitumor immunity. Clin Cancer Res. 2016;22(10):2329-2334. https://doi.org/10.1158/1078-0432.ccr-16-0224
11. Bacharier LB, Boner A, Carlsen KH, et al. Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. Allergy. 2008;63(1):5-34. https://doi.org/10.1111/j.1399-3003.2007.01586.x
12. Swanney MP, Ruppel G, Enright PL, et al. Using the lower limit of normal for the FEV1/FVC ratio reduces the misclassification of airway obstruction. Thorax. 2008;63(12):1046-1051. https://doi.org/10.1136/thx.2008.098483
13. Staples KJ, Nicholas B, McKendry RT, et al. Viral infection of human lung macrophages increases PD-L1 expression via IFN-beta. PLoS One. 2015;10(3):e0121527. https://doi.org/10.1371/journal.pone.0121527
14. Friedrich J, Heim L, Trufa DI, et al. STAT1 deficiency supports PD-L1 signaling resulting in dysfunctional TNFalpha mediated immune responses in a model of NSCLC. Oncotarget. 2018;9(8):37157-37172. https://doi.org/10.18632/oncotarget.26441
15. Bray C, Bell LN, Liang H, et al. Erythrocyte sedimentation rate and C-reactive protein measurements and their relevance in clinical medicine. WMJ. 2016;115(6):317-321.
16. Chew KS. What’s new in Emergencies Trauma and Shock? C-reactive protein as a potential clinical biomarker for influenza infection: More questions than answers. J Emerg Trauma Shock. 2012;5(2):115-117. https://doi.org/10.4103/0974-2700.96477
17. Wang XF, Lei Y, Chen M, Chen CB, Ren H, Shi TD. PD1/PD11 and CD28/CD80 pathways modulate natural killer T cell function to inhibit hepatitis B virus replication. J Viral Hepat. 2013;20(Suppl 1):27-39. https://doi.org/10.1111/jvh.12061

SUPPORTING INFORMATION
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