

# Rhinovirus inhibits IL-17A and the downstream immune responses in allergic asthma

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The proinflammatory cytokine interleukin-17A (IL-17A) is known to mediate antimicrobial activity, but its role during rhinovirus (RV) infections and in asthma needs further investigation. Therefore, we addressed the role of IL-17A during allergic asthma and antiviral immune response in human and murine immunocompetent cells. In this study we found that asthmatic children with a RV infection in their upper airways have upregulated mRNA levels of the antiviral cytokine *interferon type I (IFN)- $\beta$*  and the transcription factor *T-box 21 (TBX21)* and reduced levels of IL-17A protein in their peripheral blood mononuclear cells (PBMCs). We also found that IL-17A inhibited RV1b replication in infected human lung epithelial cells A549. Furthermore, by using gene array analysis we discovered that targeted deletion of *Il17a* in murine lung CD4<sup>+</sup> T cells impaired *Oas1g* mRNA downstream of *Irfn $\beta$* , independently from RV infection. Additionally, in PBMCs of children with a RV infection in their nasopharyngeal fluid *OAS1* gene expression was found downregulated. Finally RV1b inhibited IL-17A production in lung CD4<sup>+</sup> T cells in a setting of experimental asthma. These results indicate that the RV1b inhibits IL-17A in T helper type 17 cells and IL-17A clears RV1b infection in epithelial cells. In both cases IL-17A contributes to fend off RV1b infection by inducing genes downstream of interferon type I pathway.

## INTRODUCTION

Asthma bronchiale is a worldwide common chronic disease of the airways that affects 300 million children and adults and its incidence was found to be associated with viral infection in the upper airways, especially in children.<sup>1–4</sup> Human rhinovirus (RV) is a member of the Picornaviridae family of viruses. RVs of the minor subgroup (e.g., RV1b) adhere to proteins of the low-density lipoprotein receptor family. The primary entrance of RV is the upper respiratory tract, where it attaches to its receptors on epithelial cells.<sup>5,6</sup> The infected cells then release distress signals like chemokines and cytokines.<sup>7–11</sup>

Interleukin-17A (IL-17A) is a cytokine with proinflammatory functions, whose role in asthma is not completely understood. It is produced by T helper type 17 (Th17) cells and it is also associated with autoimmune diseases.<sup>12</sup> IL-17A was found upregulated in moderate to severe asthma where it contributes, e.g., to accumulation of neutrophils in the

airways.<sup>13</sup> Schnyder-Candrian *et al.*<sup>14</sup> showed that exogenous rmIL-17A during allergen challenge decreased airway hyperresponsiveness (AHR). However, IL-17A released by gamma delta cells was also shown to be important for the development of asthma.<sup>15</sup> IL-17A is also involved in mucosal and epithelial host defense against fungi and extracellular bacteria.<sup>16,17</sup> Furthermore, Th17 cells and IL-17A seem also to modulate immune pathophysiology of viral infections.<sup>18</sup>

The 2'-5' oligoadenylate synthetase (OAS) pathway functions as an innate host defense in response to viral infections. Its expression is upregulated by type I interferons. Via polymerization of ATP into short 2'-5'-linked oligomers (2-5A), OAS1 activates latent ribonuclease L (RNaseL), which in turn cleaves viral and cellular single-stranded RNAs.<sup>19–21</sup>

Given that there is an ongoing clinical trial to block IL-17A in asthma, the role of this cytokine in the context of RV infection needs to be clarified.<sup>22,23</sup>

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## RESULTS

**Clinical outcome of the cohorts of children analyzed in this study**

In this study, we analyzed 17 control children and 20 children with asthma. The clinical data of these cohorts of children are reported in **Supplementary Tables S1 and S2** online. The average age of controls and cases was 4.8 years ( $\pm 0.2$  years). In total, 70.6% of the control children and 60% of the asthmatic children were males and thus 29.4% of the controls and 40% of children with asthma were females. By rating the severity of the disease according to Global initiative for Asthma guidelines (2005), 60% of the children have intermittent, 25% mild persistent, and 10% moderate persistent asthma. In 65% of the cases a viral infection was a triggering factor for the development of the disease. A combined treatment with steroid and non-steroid drugs was used by 50% of children with asthma. In 45% of the cases the asthma was controlled and partially controlled, respectively (see **Supplementary Table S1**). Analysis of the lung function of cases and controls showed that 76.5% of controls and only 40% of the cases had a FEV1 above 100%. Both, control and asthmatic children, suffered from several upper respiratory infections (e.g., common cold) during the last 12 months before they were included in the PreDicta (post-infectious immune reprogramming and its association with persistence and chronicity of respiratory allergic diseases) study. By contrast, children with asthma were more susceptible to infections of the lower respiratory tract such as pneumonia (50% compared with 5%) (see **Supplementary Table S2**). This can also be seen in **Figure 1a**, where the average number of lower respiratory infections during the last 12 months was significantly increased in cases compared with control children. Furthermore, control children as well as children with asthma suffered more often from upper respiratory infections than lower respiratory infections during the last 12 months before the time of recruitment (**Figure 1a**).

**RV infection in the upper airways is associated with increased type I *IFNB* and *TBX21* mRNA expression but decreased IL-17A protein in PBMCs of preschool asthmatic children**

Within the PreDicta study we collected nasal pharyngeal fluid (NPF) for the analysis of RV infection (see **Supplementary Figure S1a and b**) in the cohort of children described above, at the time of the recruitment into the study. Furthermore, at the same time point, whole blood was drawn and PBMCs were isolated for further analysis (see **Supplementary Figure S1a and c**).

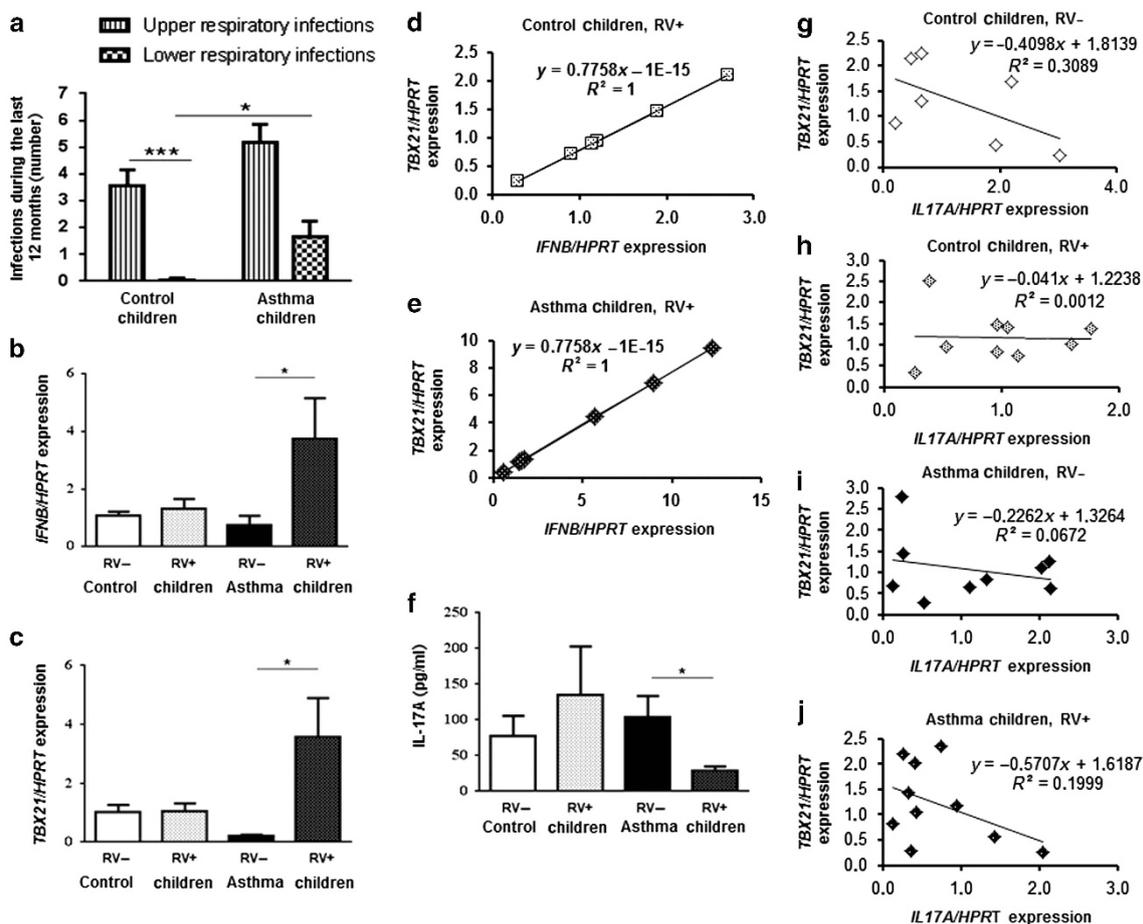
We next further classified our two cohorts of children (healthy and asthmatics) into four groups: control and asthmatic children, whose NPF was found to be positive or negative for local RV colonization. Here, we detected an increased expression of the antiviral cytokine type I interferon- $\beta$  (*IFNB*) in the PBMCs of children with asthma and a positive RV test in their NPF at the time of recruitment (RV +; **Figure 1b**). Furthermore, we found that *TBX21*, the main transcription factor for IFN $\gamma$ <sup>24</sup> and an inhibitor of

IL-17A,<sup>25</sup> was upregulated in the PBMCs of these children (**Figure 1c**). Finally, we found a perfect positive correlation between *TBX21* and *IFNB* mRNA production in the PBMCs of these children with a RV infection in their NPF (**Figure 1d and e**). Consistent with the latter findings, the PBMCs of these children also released significantly less IL-17A compared with those isolated from asthmatic children without RV infection in their nasopharyngeal fluid (**Figure 1f**), at the same time point. Furthermore, by correlating the gene expression levels of *TBX21* and *IL17A* in whole-blood samples (**Figure 1g–j**), we could observe an inverse correlation between these two factors in control children without a viral infection in the upper airways (**Figure 1g**). In control children, the presence of RV in their NPF abolished this negative correlation (**Figure 1h**). By contrast, children with asthma and no RV infection in the upper airways showed only a slight inverse correlation between *TBX21* and *IL17A* (**Figure 1i**), whereas the negative correlation was found in children with asthma and an additional viral infection in their NPF (**Figure 1j**).

The involvement of the RV in the regulation of these genes became even clearer by analyzing these markers without taking into account a viral infection in the upper airways. In this case, we could not observe any significant difference between control and asthmatic children (see **Supplementary Figure 1d–g**). Taken together these data demonstrate that a RV infection in the upper airways corresponds with an increase of *IFNB* and *TBX21* mRNA and with a suppression of IL-17A in asthmatic children.

**IL-17A inhibited RV1b replication in infected lung epithelial cells**

As mentioned above, *OAS1* is a key factor in the antiviral immunity and its transcription is induced by type I IFN signaling. Therefore, we also analyzed *OAS1* mRNA expression in the PBMCs of the PreDicta children at our department (MP-UK-ER). Here, we observed a significant downregulation of *OAS1* gene expression in the PBMCs of children with asthma and an additional RV infection in the upper airways compared with asthmatic children without a viral infection (**Figure 2a**). As both factors, *OAS1* and IL-17A, were decreased in asthmatic children with a RV infection in the upper airways, we thought about a direct impact of IL-17A on *OAS1* expression. Therefore, we started to analyze the epithelial cell line A549, as airway epithelial cells are the first cell type encountered by infectious agents entering the airways. We then asked about the role of IL-17A on RV1b infection in lung epithelial cells. With this aim we infected the human lung epithelial cell line A549 with RV1b and then cultured them for 24 h with increasing concentrations of rhIL-17A. Here we found a significant upregulation of *OAS1* gene expression after treating the A549 cells with 25 ng/ml of rhIL-17A (**Figure 2b**). To find out, if we could gain an even better induction of the antiviral immunity with higher concentrations of rhIL-17A, we treated the cells, after infection with RV1b, with up to 100 ng/ml rhIL-17A and concluded that 25 ng/ml of rhIL-17A were sufficient to reduce *RV1b* mRNA (**Figure 2c**). To determine whether IL-17A



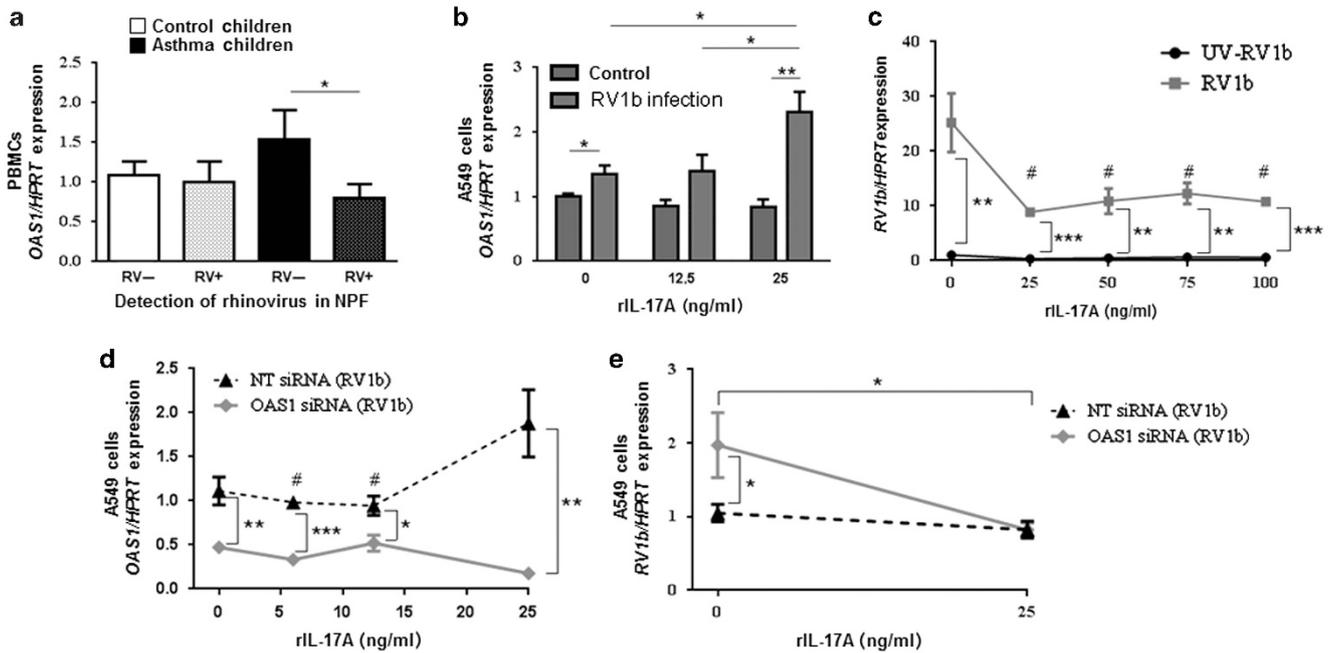
**Figure 1** Rhinovirus infection in the upper airways is associated with lower release of interleukin-17A (IL-17A) but increased expression of *IFNB* and *TBX21* in peripheral blood mononuclear cells (PBMCs) of preschool children with allergic asthma. (a) Number of upper (striped bars) and lower respiratory infections (chequered bars) during the last 12 months before PreDicta started (control children  $n=17$  and asthma children  $n=19-20$ ). (b, c, f) Control (C, white bars) and asthmatic children (A, black bars) were further subdivided with regard to rhinovirus detection (RV +, dotted bars) or (RV -) in their nasopharyngeal fluid (NPF). (b) *IFNB* mRNA expression in PBMCs after 48 h cell culture (C RV<sup>-</sup>:  $n=8$ ; C RV<sup>+</sup>:  $n=9$ ; A RV<sup>-</sup>:  $n=11$ ; A RV<sup>+</sup>:  $n=9$ ). (c) *TBX21* mRNA expression in PBMCs after 48 h cell culture (C RV<sup>-</sup>:  $n=2$ ; C RV<sup>+</sup>:  $n=6$ ; A RV<sup>-</sup>:  $n=6$ ; A RV<sup>+</sup>:  $n=7$ ). (d, e) Correlation of *IFNB* and *TBX21* mRNA expression in PBMCs after cell culture for 48 h of control children (d;  $n=6$ ) and asthmatic children (e;  $n=7$ ) who were RV +. (f) IL-17A ELISA in supernatants of PBMCs after 24 h cell culture (C RV<sup>-</sup>:  $n=4$ , C RV<sup>+</sup>:  $n=2$ , A RV<sup>-</sup>:  $n=8$ , A RV<sup>+</sup>:  $n=4$ ). (g-j) Correlation of *TBX21* and *IL17A* mRNA expression in whole-blood samples of control children and children with asthma: C, RV - (g;  $n=7$ ), C, RV + (h;  $n=9$ ), A, RV - (i;  $n=9$ ) and A, RV + (j;  $n=10$ ). Data are provided as mean  $\pm$  s.e.m. \*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.001$  (Student's *t*-test).

decreased RV1b expression via OAS1, we first treated A549 cells with OAS1 siRNA or non-targeting (NT) siRNA, infected them with RV1b, and afterwards cultured these cells with increasing concentrations of rhIL-17A. In these experiments, we also analyzed the impact of IL-17A concentrations inferior to 25 ng/ml on *OAS1* gene expression. We could only observe a significant increase of *OAS1* mRNA expression in A549 cells infected with RV1b and treated with NT siRNA and 25 ng/ml rhIL-17A compared with A549 cells infected with RV1b and treated with NT siRNA and lower amounts of rhIL-17A (Figure 2d and see Supplementary Figure S2). We could also observe that this effect was IL-17A dependent and RV1b independent, as we saw a similar induction after infection with UV-RV1b (see Supplementary Figure S2). Next, we measured *RV1b* mRNA expression in A549 cells infected with RV1b and treated with OAS1 siRNA and 25 ng/ml rIL17A. We found out that IL-17A had also in the absence of OAS1 an impact on viral

replication, as we observed a significantly downregulation of *RV1b* mRNA expression in A549 cells transfected with OAS1 siRNA and 25 ng/ml rhIL-17A, indicating that IL-17A influences viral replication also by affecting other antiviral yet unidentified factors (Figure 2e). In conclusion, these experiments show that antiviral properties of IL-17A are linked at least in part to OAS1 in lung epithelial cells.

#### Analysis of *Il17a*<sup>(-/-)</sup> mice in a murine model of allergic asthma

To investigate the role of *Il17a* during antiviral immune responses in asthma, we first analyzed wild-type (wt) and *Il17a*<sup>(-/-)</sup> mice in a murine model of allergic asthma (see Supplementary Figure S3). In this model, we found that lung CD4<sup>+</sup> T cells secreted significantly increased amounts of IL-17A when purified from the lung of OVA-sensitized mice (see Supplementary Figure S3a). Moreover, by using an invasive



**Figure 2** Interleukin-17A (IL-17A) inhibited RV1b replication in epithelial cells and induced *OAS1*. (a) Control (C, white bars) and children with asthma (A, black bars) were further subdivided with regard to rhinovirus detection (RV +, dotted bars) or (RV -) in their nasopharyngeal fluid *OAS1* mRNA expression in peripheral blood mononuclear cells (PBMCs) after cell culture for 48 h (C RV<sup>-</sup>: *n* = 8, C RV<sup>+</sup>: *n* = 9, A RV<sup>-</sup>: *n* = 11, A RV<sup>+</sup>: *n* = 9). (b) *OAS1* mRNA expression in A549 cells after infection with RV1b or control medium and responding to different doses of rIL-17A (*n* = 3 per group). (c) Infection of A549 cells with RV1b or UV-RV1b and subsequent cell culture for 24 h with different concentrations (0/25/50/75/100 ng/ml) of rIL-17A (*n* = 3 per group). (d–e) Transfection of A549 cells with either *OAS1* siRNA or non-targeting (NT) siRNA. Subsequent infection with RV1b or UV-RV1b and cell culture responding to different doses of rhIL-17A (d: 0/6/12.5/25 ng/ml rhIL-17A; e: 0/25 ng/ml rhIL-17A). (d) *OAS1* mRNA expression in A549 cells after infection with RV1b and transfection with either NT or *OAS1* siRNA (*n* = 3 per group). (e) *RV1b* mRNA expression in A549 cells after infection with RV1b and transfection with *OAS1* or NT siRNA (*n* = 7 per group). Data are provided as mean  $\pm$  s.e.m. \**P*  $\leq$  0.05; \*\**P*  $\leq$  0.01, \*\*\**P*  $\leq$  0.001. c: #*P* < 0.05 compared with cells infected with RV1b not stimulated with rhIL-17A (Student's *t*-test). d: #*P* < 0.05 compared with NT siRNA stimulated with 25 ng/ml rhIL-17A (Student's *t*-test).

plethysmography method, we found a significant increase in AHR in *Il17a*<sup>(-/-)</sup> mice at lower doses of methacholine (MCh), whereas at higher doses of MCh the wild-type littermates showed an increased AHR as compared with the *Il17a*-deficient mice (see **Supplementary Figure S3b**). Consistent with a role of the Th2-type cytokines in allergic asthma, the Th2 cytokine IL-4 was found to be significantly induced in the airways of OVA-sensitized *Il17a*<sup>(-/-)</sup> mice as compared with wild-type littermates (see **Supplementary Figure S3c**). Consistent with this increase in IL-4 OVA-treated *Il17a*<sup>(-/-)</sup> mice showed a significant decrease of IFN- $\gamma$  in BALF (see **Supplementary Figure S3d**). IL-5 and IL-13, two cytokines produced by Th2 cells and by the innate ILC2 cells,<sup>26,27</sup> were found to be similarly induced in the airways of OVA-sensitized wild-type and *Il17a*-deficient mice (see **Supplementary Figure S 3e and f**). Consistent with the levels of IL-5 in the airways, the amount of eosinophils was equivalent in OVA-sensitized *Il17a*<sup>(-/-)</sup> and wild-type mice (see **Supplementary Figure S4a**). By contrast, *Il17a*<sup>(-/-)</sup> OVA-sensitized mice have reduced neutrophils in their airways (see **Supplementary Figure S4b**) and a reduced number of mucus producing cells in the airways as compared with their wild-type littermates (see **Supplementary Figure S4c**). Both these latter components are known to be involved in the

clearance of infectious microorganisms.<sup>28,29</sup> IL-4 is known to induce IgE immunoglobulin class switching in B cells.<sup>30</sup> However, in our model system, the serum levels of IgE in OVA-sensitized *Il17a*<sup>(-/-)</sup> mice were comparable to those measured in the wild-type littermates (see **Supplementary Figure S4d**). We further investigated *Batf* mRNA expression, a transcription factor<sup>31</sup> that is expressed in lymphocytes and is also involved in immunoglobulin rearrangements and thus in IgE induction in allergic asthma.<sup>32</sup> Furthermore, it could be shown that BATF is increased in asthma.<sup>32</sup> Consistently in this murine model of allergic asthma *Batf* mRNA was found to be increased in allergic asthma in an *Il17a*-independent manner (see **Supplementary Figure S4e**).

#### Antiviral *Oas1* genes are inhibited in lung CD4<sup>+</sup> T cells obtained from OVA-sensitized *Il17a*<sup>(-/-)</sup> mice

Aeroallergen sensitization is one of the strongest risk factors for asthma.<sup>33</sup> However, viral infection has also been emerged as a crucial factor in the pathogenesis of this disease.<sup>34</sup> To understand the role of IL-17A in the regulation of different genes that are involved in the response to viral infections, we used a gene array approach in our study. Thus, we sorted out lung CD4<sup>+</sup> T cells from OVA-sensitized wild-type and *Il17a*<sup>(-/-)</sup> mice and isolated total RNA and performed gene

array analysis. By using this method, we found in Il17a-deficient lung CD4<sup>+</sup> T cells a significant downregulation of antiviral Oas family members such as *Oas1a*, *Oas1c*, and *Oas1g* as well as *Cxcr3* and *Rnasel* (see **Supplementary Figure S5a and b**) and an upregulation of proinflammatory genes such as *Il6*, *Il13*, *Nfil3*, and *PDC-Trem*. By intracellular flow cytometry analysis we also found an upregulation of IL-13 in CD4<sup>+</sup> T cells of naïve Il17a<sup>(-/-)</sup> mice (see **Supplementary Figure S5c**). The downregulation of *Oas1a* and *Oas1g* was verified by real-time PCR in lung CD4<sup>+</sup> T cells isolated from naïve and OVA-sensitized wild-type and Il17a<sup>(-/-)</sup> mice (see **Supplementary Figure S5d and e**).

We also observed a significant decrease of *Oas1g* mRNA in whole-lung tissue and draining lymph nodes of naïve and OVA-sensitized Il17a<sup>(-/-)</sup> mice (see **Supplementary Figure S5g**). Furthermore, in lung CD4<sup>+</sup> T cells *Ifnb* mRNA, which is the inducing factor of *Oas1g*, was found significantly downregulated in naïve Il17a<sup>(-/-)</sup> mice. Treatment with OVA resulted indeed in an inhibition of the *Ifnb* mRNA expression in wild type as well in Il17a<sup>(-/-)</sup> mice, but we could not detect significant differences within this group any longer (see **Supplementary Figure S5h**).

Taken together, we observed defective expression of *Oas1g* and *Ifnb*, two genes involved in the antiviral immune response, in the lung of Il17a-deficient mice in a setting of allergic asthma. These data indicate a genetic control of Il17a on the antiviral gene *Oas1*.

### RV1b infection downregulated IL-17A in lung CD4<sup>+</sup> T cells isolated from OVA-sensitized wild-type mice

It is already known that lymphocytes express the low-density lipoprotein receptor, which is the portal of entry for minor-group RVs such as RV1b. Furthermore, it could also be demonstrated that RV, in contrast to RSV, is able to directly infect and activate CD4<sup>+</sup> T cells.<sup>35-37</sup> To address the role of IL-17A during RV infection in asthma, we infected lung CD4<sup>+</sup> T cells obtained from OVA-sensitized wild type and Il17a<sup>(-/-)</sup> mice with RV1b and cultured them for 24 h (**Figure 3a**). By analyzing these cells we could observe that Il17a-deficient lung CD4<sup>+</sup> T cells from naïve mice express significantly more *Ldlr* than the corresponding wt mice. Furthermore, confrontation with the allergen OVA resulted in a significant upregulation of the expression of this receptor in CD4<sup>+</sup> T cells of wt mice, whereas Il17a-deficient CD4<sup>+</sup> T cells show a downregulation of the *Ldlr* mRNA expression (**Figure 3b**). By using PCR analysis (see **Supplementary Figure S6**) we could confirm the infection with RV1b and after quantification of the PCR bands we found out that lung CD4<sup>+</sup> T cells of naïve Il17a<sup>(-/-)</sup> mice are more susceptible to viral infection than lung CD4<sup>+</sup> T cells of naïve wt mice (**Figure 3c**). Moreover, we could observe a significant upregulation of the viral load in lung CD4<sup>+</sup> T cells obtained from OVA-sensitized wt mice compared with the cells of naïve wt mice, whereas there was no change in the viral load in lung CD4<sup>+</sup> T cells of OVA-sensitized Il17a<sup>(-/-)</sup> mice, neither to OVA-sensitized wt cells nor to cells of naïve Il17a-deficient mice (**Figure 3c**). The increase of viral load observed

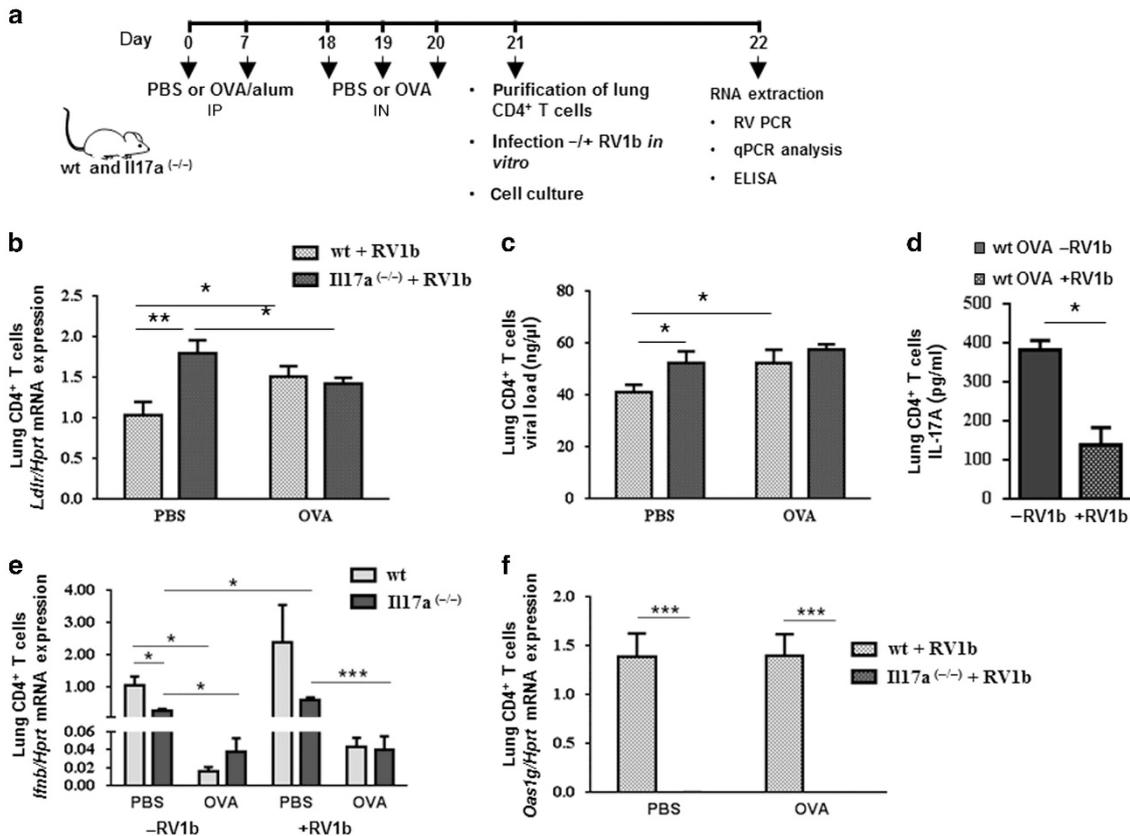
in CD4<sup>+</sup> T cells of OVA-sensitized wt mice could be due to the reduced release of IL-17A by these cells after infection with RV1b (**Figure 3d**).

Furthermore, the deficit of *Ifnb* mRNA expression, which was seen after confrontation with the allergen OVA in wild-type and Il17a<sup>(-/-)</sup> lung CD4<sup>+</sup> T cells, could not be restored after infection with RV1b (**Figure 3e**). Tallying with this, we still observed the defect in *Oas1g* gene expression in Il17a-deficient lung CD4<sup>+</sup> T cells after viral infection (**Figure 3f**). Taken together, these data indicate that RV1b targets Th17 cells in the lung of OVA-sensitized mice.

The described data lead to the suggestion that in the case of a RV infection in the upper airways, IL-17A inhibits the expression of *LDL-R* and thus the cellular entry of the RV in airway epithelial cells (**Figure 4**). Furthermore, by inducing the expression of IFN- $\beta$  and OAS1, IL-17A also contributes to the suppression of viral replication. Subramaniam and colleagues<sup>38,39</sup> observed that IL-17A induced—amongst others—in a time-dependent manner the tyrosine phosphorylation of JAK1 and TYK2 as well as of STAT1 and STAT2. This might be the underlying signaling mechanism by which IL-17A induces OAS1 gene expression. Furthermore, it is already known that IL-17A activates nuclear factor- $\kappa$ B<sup>40</sup> and this transcription factor is involved in the transcription of IFN- $\beta$ ,<sup>41</sup> which in turn leads to the activation of antiviral factors such as OAS1 via JAK1/TYK2, STAT1/2 and IRF9. But our data also suggest that RV is able to inhibit the release of IL-17A and therefore escaping the immune system (**Figure 4**). To elucidate these molecular mechanisms in more detail, further investigations are needed.

### DISCUSSION

In the present study we discovered a defect of IL-17A as well as interferon-induced OAS1, an antiviral enzyme, situated downstream of IFN- $\beta$  (**Figure 4**), in the PBMCs of pre-school asthmatic children with RV infection in the upper airways. OAS1 activates RNaseL, which mediates RNA virus degradation.<sup>19,20</sup> The reduced release of IL-17A by the PBMCs of these children might be due to the induction of *TBX21* in the PBMCs of these children, a transcription factor that is known to negatively regulate IL-17A.<sup>25</sup> Furthermore, we found that IL-17A inhibited RV replication in human lung epithelial cell line infected with RV1b. We could also show that IL-17A upregulated *OAS1* in this setting. This IL-17A/OAS1 pathway seems to be independent of IFN $\beta$ , as we found increased expression of *IFNB* in children with asthma and an additional RV infection in their upper airways, whereas IL-17A and *OAS1* were reduced. This observation is in line with previous findings on increased levels of *IFNB* in PBMCs after infection with RV *in vitro*.<sup>42,43</sup> In contrast to these results in PBMCs, in bronchial epithelial cells and in the lung epithelial cell line A549 it has been previously reported that RV inhibits type I interferons.<sup>43-46</sup> Therefore, it is possible that epithelial cells, which are the first target of the RV, exhibit different immune response as compared with PBMCs. Finally, it is also possible that the usage of different RV strains influences the overall results.



**Figure 3** Infection of lung CD4<sup>+</sup> T cells with RV1b. **(a)** Experimental design: Induction of allergic asthma and subsequent purification of lung CD4<sup>+</sup> T cells with magnetic anti-CD4 beads from naïve and asthmatic wt and Il17a-deficient mice and successive infection with RV1b *in vitro* and cell culture for 24 h. **(b)** *Ldlr* mRNA expression in lung CD4<sup>+</sup> T cells of wt and Il17a-deficient mice after infection with RV1b ( $n=4$ ). **(c)** PCR to detect and quantify the rhinovirus infection in lung CD4<sup>+</sup> T cells from OVA-sensitized wild-type mice with and without RV1b infection ( $n=2-4$ ). **(d)** IL-17A protein in supernatants of lung CD4<sup>+</sup> T cells from OVA-sensitized wild-type mice with and without RV1b infection ( $n=2-4$ ). **(e)** *Ifnb* mRNA expression in lung CD4<sup>+</sup> T cells of wt and Il17a<sup>-/-</sup> mice with or without infection with RV1b ( $n=2-4$ ). **(f)** *Oas1g* mRNA expression in lung CD4<sup>+</sup> T cells of wt and Il17a<sup>-/-</sup> mice after infection with RV1b ( $n=2-4$ ). \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$  (c-f: ANOVA, b: Student's *t*-test).

We also observed increased *TBX21* mRNA expression in PBMCs of children with asthma and a RV infection in their upper airways. We previously demonstrated that this Th1 specific transcription factor inhibits IL-17A.<sup>25</sup> In addition, we could also show that *TBX21* and *IFNB* expression perfectly correlated in healthy and asthmatic children with a viral infection in the upper airways.

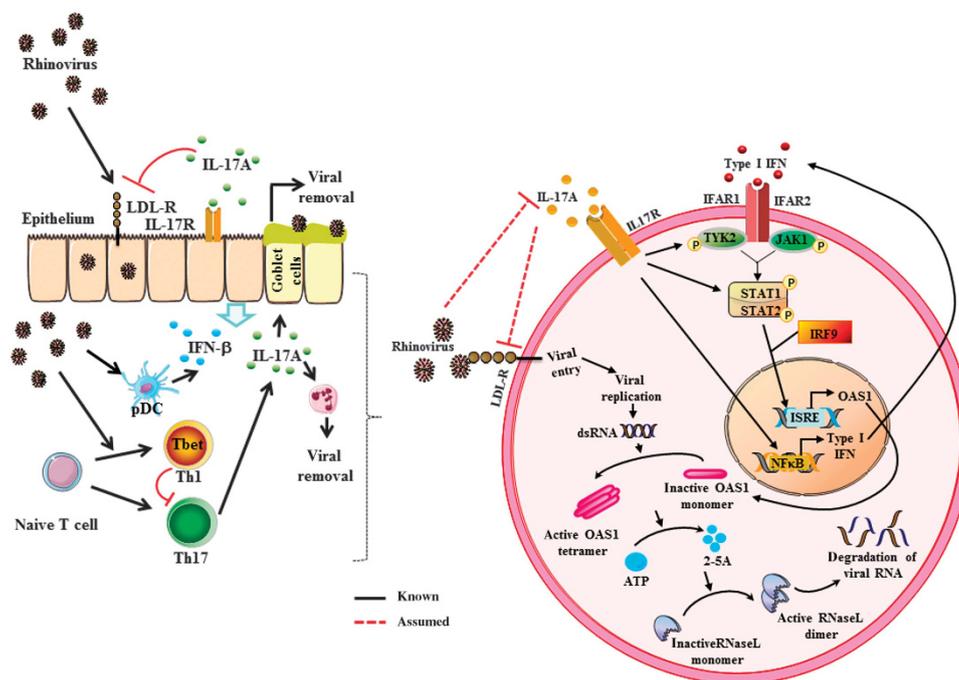
By using gene array, we found that Il17a-deficient lung CD4<sup>+</sup> T cells isolated from OVA-sensitized mice do not express *Oas1g* and consequently the expression of *RNase1* which is downstream of *Oas1* and responsible for viral RNA degradation was also found downregulated. This might be due to either a direct or indirect effect secondary to the inhibited *Ifnb* expression seen in these mice. This might also lead to the assumption that IL-17A is involved in the induction of type I interferons. This is in line with findings of Wang and colleagues in BXD2 mice, where they could show that IL-17A favors the expression of *Ifnb*.<sup>47,48</sup> Further experiments need to be performed to demonstrate this.

The influence of IL-17A on AHR is controversially discussed. These observations seem to depend on the time point of IL-17A release.<sup>49</sup> By using the invasive measurement of AHR, we found increased responses to low doses of

MCh in OVA-sensitized Il17a-deficient mice as compared with wild-type littermates. At higher doses of MCh OVA-sensitized wild-type mice showed an increased hyperreactivity than the Il17a-deficient mice. The reason of this dual response is at the moment not clear. However, we found increased IL-4 levels and decreased IFN- $\gamma$  in the lung of Il17a-deficient mice which could explain the different responses to MCh. It is possible that IL-4 is acting on the AHR at low doses of MCh and other not yet analyzed factors such as IL-33 and IL-25.<sup>50-53</sup> In addition, it is already known that IL-17A has an effect on IL-13-induced AHR.<sup>54</sup> Therefore, the induced airway reactivity at low doses seen in the Il17a-deficient mice might be due to the increased combined amounts of IL-13 and IL-4.<sup>26</sup>

Additionally, we found less PAS<sup>+</sup> cells and airway neutrophils in the absence of Il17a. These two components are known to be involved in the clearance of pathogens.<sup>28,29</sup> Therefore, this mechanism of host defence is also impaired in the absence of IL-17A.

It is already known that RV could bypass antigen presentation and could directly infect T cells.<sup>37</sup> We thus next ask whether the RV targets Th17 cells in asthma. We found that



**Figure 4** Interleukin-17A (IL-17A) mediated regulation of rhinovirus-induced inflammation. The IFN-induced OAS1-RNaseL antiviral pathway: Type I IFNs bind to the heterodimer of IFN $\alpha$  receptor 1 (IFNAR1) and IFNAR2 which initiates the signal transduction via the tyrosine kinases JAK1 (Janus kinase 1) and TYK2 (Tyrosine kinase 2). In the nucleus, OAS1 (2'5'-oligoadenylate synthetase 1) is transcribed upon induction of IFN-stimulated response elements (ISREs). In the cell cytoplasm OAS1 protein accumulates as an inactive monomer. Upon binding of double-stranded RNA (dsRNA), the enzyme oligomerizes to form a catalytic active tetramer. This active form synthesizes 2'5' oligoadenylates (2-5A) using ATP as substrate. 2-5A binds to inactive RNaseL and triggers the dimerization of monomers. RNaseL then degrades RNA of viral and also cellular origin. This leads to the inhibition of viral propagation.<sup>20,61–63</sup> We assume that IL-17A could induce IFN- $\beta$  and OAS1 expression and inhibits low-density lipoprotein receptor expression, whereas in turn, rhinovirus could suppress IL-17A.

infection of lung CD4<sup>+</sup> T cells with RV1b resulted in a significant reduction of IL-17A. These results indicate that Th17 cells could be the target of RV in asthma. Consistent with this finding, lung CD4<sup>+</sup> T cells from OVA-sensitized wild-type mice infected with RV1b have the same viral load as the infected lung CD4<sup>+</sup> T cells from Il17a-deficient mice. These observations could be due to particular expression of *Ldlr*, which was found upregulated in lung CD4<sup>+</sup> T cells of naïve Il17a-deficient mice, whereas confrontation with the allergen promoted the *Ldlr* mRNA expression in wt cells but inhibited it in Il17a-deficient cells. Furthermore, we could show by analysing CD4<sup>+</sup> T cells of naïve and OVA-sensitized mice that treatment with OVA negatively regulated *Ifnb* mRNA expression independently of infection with RV1b.

Epithelial cells are the first cells that the RV encounters after inhalation and the RV has a positive tropism for this cell type.<sup>6,55</sup> These results clearly demonstrate a crucial mechanism on how the RV escapes the immune system by inhibiting IL-17A production by Th17 cells and thus depriving epithelial cells from IL-17A resulting in reduction of OAS1 and thus creating a good cellular microenvironment where the virus can replicate. Taken together, these results demonstrate that the RV infection targets Th17 cells in the airways of OVA-sensitized mice. Similarly to what we observed in the PBMCs of asthmatic children, RV1b reduces IL-17A production in infected CD4<sup>+</sup> T

cells from OVA-sensitized mice. This results in IL-17A deprivation which cannot anymore signal in cells of the innate immune response like epithelial cells. We further demonstrated that IL-17A via OAS1 activation shreds the RV RNA which replicates preferentially in epithelial cells. These findings have important implication for the treatment of RV-induced asthma in children.

## METHODS

**Human studies: detection of OAS1, TBX21 and IFNB mRNA and IL-17A protein in PBMCs.** Within the PreDicta study (Post-infectious immune reprogramming and its association with persistence and chronicity of respiratory allergic diseases) we analyzed two cohorts of children with and without asthma at the age of 4–6 years. The study was approved by the ethics committee of the Friedrich-Alexander University Erlangen-Nürnberg, Germany (Re-No 4435). Informed consent was obtained from the parents of all participants of this study (Study PreDicta, Registration number “Deutsches Register Klinischer Studien” (German Clinical Trials Register) DRKS00004914). We isolated PBMCs from the whole blood using Ficoll and density gradient centrifugation. After cell culture we analyzed them by ELISA and quantitative PCR. The clinical outcomes of the recruited children in this study are reported in **Supplementary Table S1 and S2**.

**Nasopharyngeal fluid collection with swab and RV detection.** For the detection of the RV in the upper airways, a nasopharyngeal specimen from control children and asthmatic children was collected

using a pernasal applicator swab, which has a tip with flocked soft nylon fiber (E-Swab 482CE; Copan, Brescia, Italy).

RV detection was performed at the Department of Virology, University of Turku (Finland). Therefore, nucleic acids were extracted using easyMag extractor (BioMeriex, Marcy l'Etoile, France) from 200 µl of the medium according to the manufacturer's instruction. An *in house* PCR method was used to detect enteroviruses, RV s and respiratory syncytial viruses as described earlier.<sup>56</sup> A commercial test kit (Anyplex II RV16 Detection; Seegene, Seoul, Korea) was used to detect 16 respiratory viruses (adenovirus; influenza A and B viruses; parainfluenza virus 1, 2, 3, and 4; RV A, B, and C; respiratory syncytial virus A and B; bocavirus 1,2,3,4; coronavirus 229E, NL63, and OC43; metapneumovirus; and enteroviruses) (see **Supplementary Figure S1c**).

**Culture of human A549 lung epithelial cells and murine lung CD4<sup>+</sup> T cells and infection with RV1b.** To infect the cells with RV1b, cells were shook for 1 h at room temperature with RV1b suspension. RV1b was grown as previously described.<sup>57</sup> Comparable treatment with UV-irradiated RV1b or medium served as a control. After RV infection, the cells were washed with medium. A549 cells were cultured for 24 h with medium alone or with an increasing concentration of rhIL-17A as indicated. Lung CD4<sup>+</sup> T cells were cultured without additional stimuli for 24 h.

**Mice.** Mixed gender of Il17a<sup>(-/-)</sup> mice (generously provided to us by Yoichiro Iwakura) and wild-type mice on a Balb/c genetic background were used at the age of 6–8 weeks. The mice were maintained under specific pathogen-free conditions and all experiments were undertaken with approved license (54-2532.1-2/10 from the government of Mittelfranken, Bavaria, Germany).

**OVA sensitization and challenge.** Mice received intraperitoneal (IP) injections either of phosphate-buffered saline or of 500 µg/ml ovalbumin (OVA) complexed with 10% alum on days 0 and 7 as described previously.<sup>26,58</sup> On days 18, 19, and 20 the animals were treated intranasally (IN) with phosphate-buffered saline alone or OVA in phosphate-buffered saline (2 mg OVA/ml phosphate-buffered saline in solution).

**Gene Array in purified lung CD4<sup>+</sup> T cells from wild type and Il17a deficient OVA-sensitized mice.** The gene array was performed at the Institute of Human Genetics in Erlangen. Briefly, RNA from lung CD4<sup>+</sup> T cells from OVA-sensitized Il17a<sup>(-/-)</sup> and wild-type mice was isolated, the quality control was performed and the GeneChip Gene 1.0 ST Array was used for further analysis as previously described.<sup>59</sup>

**Silencing of OAS1 in A549 cells infected with RV1b.** Lung epithelial cells A549 were transfected with OAS1 siRNA (Dharmacon ON-TARGETplus SMART pool, Lafayette, CO) and afterwards they were cultured and infected with RV1b in the presence of increasing concentrations of rhIL-17A for 24 h. For supervisory purpose and to visualize effective transfection we also transfected the cells with a non-targeting (NT) control (Dharmacon ON-TARGETplus Control Pool, Non-Targeting pool).

**RV PCR and real-time PCR.** To verify that the infection with RV1b was successful a PCR was performed as described previously with some modifications.<sup>57,60</sup> Therefore, cDNA from infected cells and primers, which are complementary to the antisense RNA at positions 542–557 and 169–185 in the 5'-noncoding region of RV1b, called OL26 and OL27, was used. A 380-bp amplicon was generated and the samples were analysed and quantified using the QIAxcel Advanced System (Quiagen, Hilden, Germany). For detection of RV1b via real-time PCR, RNA was reverse-transcribed into cDNA. The resulting template cDNA was amplified by quantitative real-time PCR using SsoFast EvaGreen Supermix (Bio-Rad Laboratories, München, Germany) and 200 nM primers.

**Statistical analysis.** Differences were evaluated for significance ( $*P \leq 0.05$ ;  $**P \leq 0.01$ ,  $***P \leq 0.001$ ) by using one-way ANOVA or the Student's *t*-test for independent events (Excel, Microsoft, version 2003; Microsoft, Redmond, Washington), as indicated in the figure legends. Data are given as mean values  $\pm$  s.e.m.

**SUPPLEMENTARY MATERIAL** is linked to the online version of the paper at <http://www.nature.com/mi>

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#### AUTHOR CONTRIBUTIONS

A.G. and S.F. designed the experiments and A.G. performed the experiments. S.N. did the invasive measurement and analysis of the AHR. A.E. and F.F. performed the gene array analysis. T.Z. and V.O.M. were responsible for the clinical part WP1-UK-ER-of the PreDicta study in Erlangen. T.V. did the RV analysis of the NPF. N.P. provided the protocol for the *in vitro* cell infection with rhinovirus and S.T. expanded the RV and provided us a large amount to perform our complete study. S.F. and A.G. wrote the manuscript. S.F. directed the research.

#### DISCLOSURE

The authors declare no conflict of interest.

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