Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Society. W. Timens has consultant arrangements with Pfizer; has received lecture fees from GlaxoSmithKline, Chiesi, and Roche Diagnostics; and has received a grant from the Netherlands Lung Foundation. M. Van den Berge has received grants from Astra Zeneca, TEVA Pharma, GlaxoSmithKline, and Chiesi. D. S. Postma has consultant arrangements with Astra Zeneca, Chiesi, Boehringer Ingelheim, TEVA, GlaxoSmithKline, and Takeda. B. N. Melgert has received grants from the Netherlands Lung Foundation (grant no. 3.2.10.056) and the Netherlands Northern CARA Foundation. The rest of the authors declare that they have no relevant conflicts of interest.

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Role of TGF-β in anti-rhinovirus immune responses in asthmatic patients

To the Editor:

The majority of viral infections of the airways are associated with asthma exacerbations in children. Two thirds of these viral infections are caused by rhinovirus, and hospital admissions for asthma correlate with the seasonal peak of rhinovirus infections.[1]

TGF-β is a cytokine known to induce forkhead box $P3^+$ (FoxP3) regulatory T (Treg) cells and retinoic acid–related orphan receptor (ROR) γt^+ T_H17 cells in combination with IL-2 or IL-6, respectively, but also to inhibit the differentiation of T_H1 and T_H2 cells.[2]

Because TGF-β and rhinovirus infection both influence asthma exacerbation and TGF-β also induces rhinovirus replication,[3] in this study we analyzed the effect of rhinovirus infection on TGF-β and the role of TGF-β on rhinovirus infection by analyzing asthmatic and nonasthmatic preschool children recruited in the European study Post-infectious Immune Reprogramming and Its Association with Persistence and Chronicity of Respiratory Allergic Diseases (PreDicta) and a murine model of asthma. The clinical data of the analyzed cohorts of children are reported in Table E1 and in the Methods section in this article's Online Repository at www.jacionline.org. In asthmatic children, in 66.6% of the cases, a viral infection was a triggering factor for development of the disease. Rhinovirus was the most common respiratory virus detected in the airways of these children (see Table E2 in this article's Online Repository at www.jacionline.org).

To investigate the role of TGF-β in rhinovirus-induced asthma in children, we analyzed PBMCs from preschool children with and without asthma, which were cultured for 48 hours after 1 hour of *in vitro* exposure to rhinovirus 1B (RV1B) and subjected them to gene array (Fig 1, *A*, and see Tables E3-E5 in this article's Online Repository at www.jacionline.org). Because TGF-β induces Treg cells,[2] we first investigated which genes related to tolerance were significantly regulated by rhinovirus in PBMCs from these children. Here we found that in asthmatic children rhinovirus upregulated immunosuppressive genes, such as cytotoxic T lymphocyte–associated protein 4 *(CTLA4)* and indoleamine 2,3-dioxygenase *(IDO)*, programmed death ligand 1 (PD-L1; *CD274*), and interferon-induced transmembrane protein 2 (*IFITM2*; Fig 1, *B* and *C*). Consistent with the array data, we found that *IDO1* was upregulated in PBMCs of asthmatic children cultured with rhinovirus compared with those of control children (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). This regulation was found to be independent from steroids because dexamethasone significantly downregulated *IDO* in PBMCs (see Fig E1, *B*).

Because TGF-β is secreted in a latent complex consisting of 3 proteins (TGF-β, the inhibitor latency-associated protein [LAP], and the ECM-binding protein LTBP), we also analyzed these and other TGF-β–inhibitory proteins. We noticed that TGF-β–inhibitory genes, such as *TGIF2* and *LAP3*, were upregulated in rhinovirus-treated PBMCs from asthmatic children. Moreover, rhinovirus inhibited genes that cleave viruses, such as *RNASE1*, in PBMCs from children with asthma (Fig 1, *B* and *C*). By contrast, in control children rhinovirus did not significantly regulate these genes. In these children other factors were found to be significantly regulated by rhinovirus, such as lymphocyte antigen 6E (Fig 1, *D* and *E*), a protein involved in the TGF-β pathway. Moreover, we found that in PBMCs from control children, rhinovirus induced IL-32 (Fig 1, *C* and *D*). Expression of this protein is known to induce the production of IL-6 and TNF-α and might thereby modulate immune responses.[4]

In subsequent experiments we analyzed in more detail the regulation of TGF-β in a larger group of children in the same cohort. Among PBMC supernatants, TGF-β protein was detected in high amounts in untreated cell-culture supernatants in both asthmatic and control children. However, after *ex vivo* challenge with rhinovirus, TGF-β protein expression was found to be significantly decreased (Fig 2, *A*), although *TGFB* mRNA expression remained constant (Fig 2, *B*). Because rhinovirus infection suppressed TGF-β release, we assumed that rhinovirus facilitates TGF-β binding to the cell membrane, and for this reason, we could not detect it in the supernatants of rhinovirus-infected PBMCs.

To prove this concept of a viral immune escape mechanism, we analyzed the expression of *TGFBRII* in PBMCs in the presence or absence of *in vitro* rhinovirus infection. We found that PBMCs isolated from control and asthmatic children and infected with

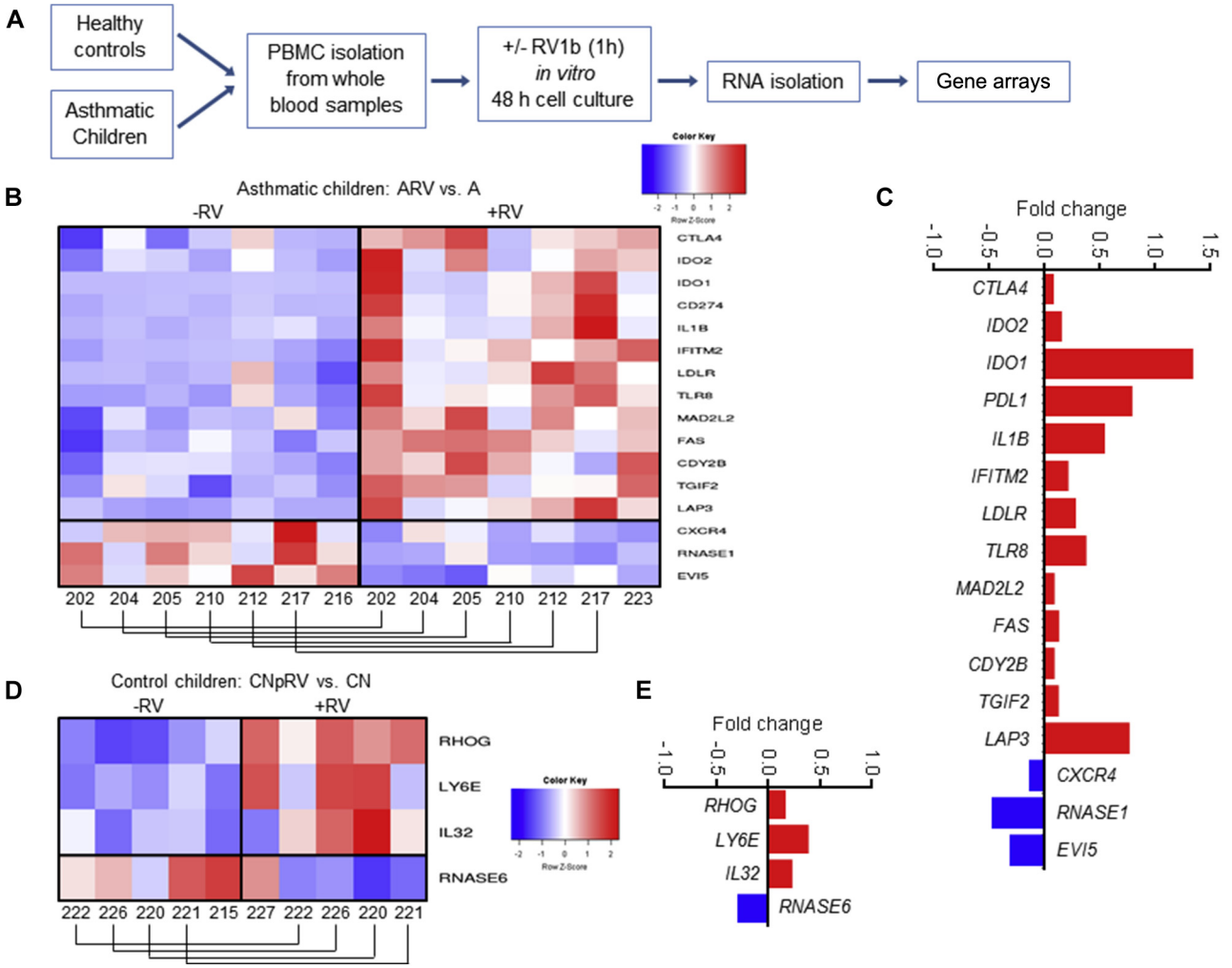

FIG 1. PBMCs from asthmatic children exposed to RV1b *in vitro* upregulated *IDO*, *PDL1*, and *LAP3*. **A,** Experimental design for RNA arrays of PBMCs cultured in the presence or absence of rhinovirus *(RV)*. **B-E,** Heat maps for asthmatic (Fig 1, *B* and *C*) and control (Fig 1, *D* and *E*) children and a differential expression analysis of the regulated genes are shown (asthma: n = 7, control: n = 5).

rhinovirus expressed increased levels of *TGFBRII* compared with the respective controls (Fig 2, *C*). This finding suggests that rhinovirus induced TGF-β receptor II expression, thus increasing TGF-β binding to the cell membrane and in this way explaining why we could not detect it in the cell supernatants.

To further analyze the influence of TGF-β signaling in molecules downstream of TGF-β, we then analyzed *FOXP3* and *RORC* levels and found that PBMCs infected *in vitro* with rhinovirus express significantly more *FOXP3* and *RORC* mRNA (Fig 2, *D* and *E*) in both control and asthmatic children.

When we analyzed the correlation of *FOXP3* and *RORC* mRNA expression, we found a positive correlation of these 2 transcription factors in rhinovirus-challenged PBMCs in both groups of children (Fig 2, *F-I*). Taken together, rhinovirus infection induced *FOXP3* and *RORC*.

We then asked whether T-box transcription factor (T-bet), a transcription factor known to regulate $T_H1/2$, Treg, and T_H17 cell development or activation,[5] could be regulated by rhinovirus in PBMCs of children with asthma. Although we previously detected decreased *TBX21* mRNA expression in asthmatic patients,[6] here we found increased *TBX21* mRNA levels in PBMCs isolated from asthmatic children after infection with rhinovirus compared with rhinovirus-infected PBMCs from control children (Fig 2, *J*). Thus *TBX21* can be upregulated in asthmatic patients during active rhinovirus infection.

IL-6 is an inflammatory cytokine that, together with TGF-β, can induce the differentiation of T_H17 cells.[2] We found an upregulation of *IL6* mRNA in control children after *in vitro* culture with rhinovirus. In contrast, asthmatic children showed a failure of such *IL6* induction (Fig 2, *K*).

By analyzing naive and asthmatic mice, we found that *in vitro* treatment of lung cells with rhinovirus increased the proportions of T_C1 cells, whereas adding TGF-β to the culture inhibited T-bet expression in $CD4^+$ T cells, as well as IDO expression in total lung cells. The experimental set up, as well as the results, are described in detail in Figs E2 and E3 in this article's Online Repository at www.jacionline.org.

In summary, these data suggest that in patients with acute rhinovirus infections, endogenous TGF-β is retained intracellularly in rhinovirus-infected cells, resulting in a

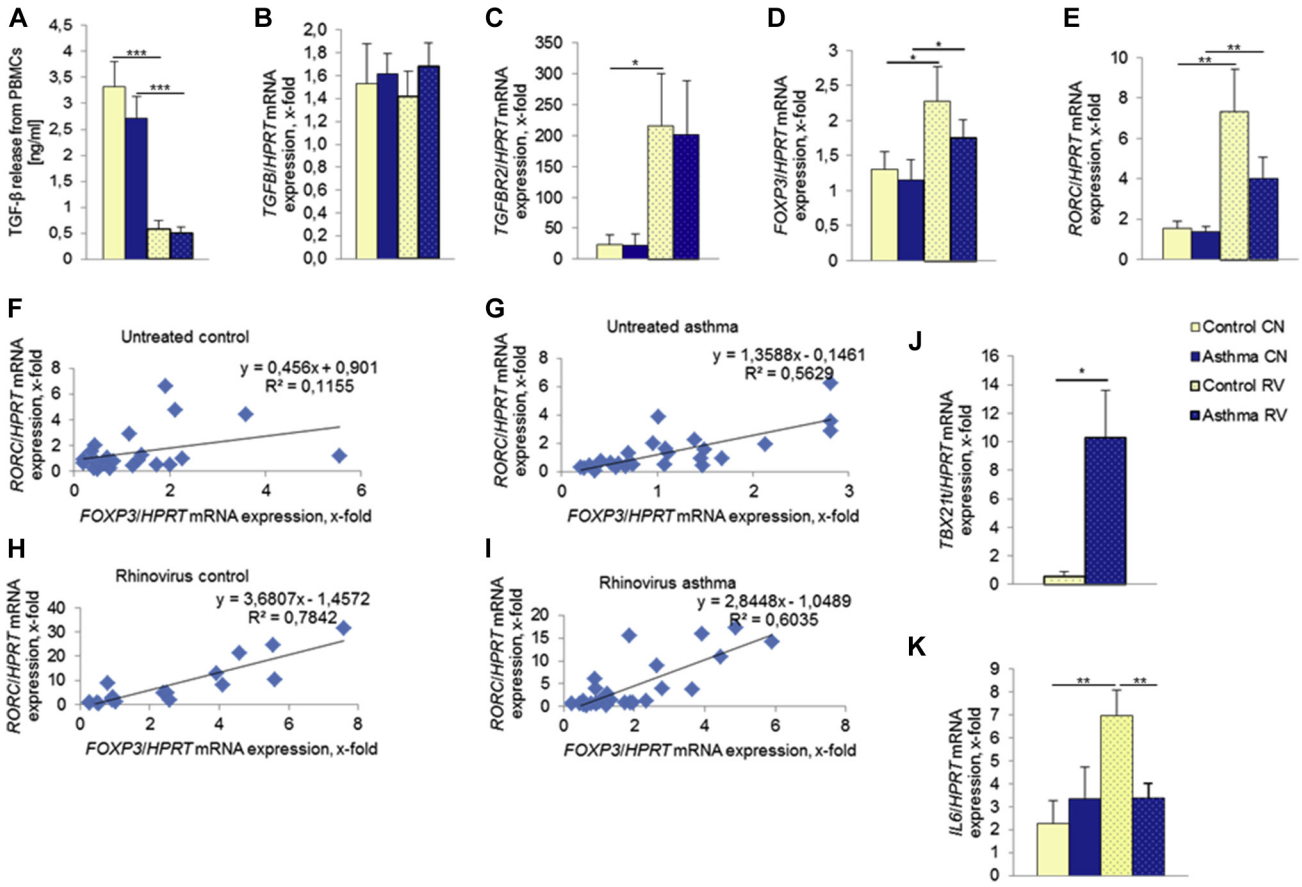

FIG 2. Rhinovirus *(RV)* inhibits TGF-β release from PBMCs isolated from healthy and asthmatic children. **A,** TGF-β1 release from PBMCs of asthmatic and nonasthmatic children with or without *in vitro* rhinovirus infection analyzed by means of ELISA (n = 26-32 children per group, B0+F4). **B-E,** Relative mRNA expression of *TGFB* (Fig 2, *B*; n = 12-20), *TGFBRII* (Fig 2, C; n =3-6), *FOXP3* (Fig 2, *D*; n = 19-31), and *RORC* (Fig 2, *E*; n = 19-31) in asthmatic and nonasthmatic children with or without *in vitro* rhinovirus infection (B0+F4) analyzed by means of real-time PCR. **F-I,** Correlation of *RORC* and *FOXP3* mRNA expression in untreated and *in vitro*–infected PBMCs from asthmatic and nonasthmatic children. **J** and **K,** Relative *TBX21* (Fig 2, *J*; n = 10-22) or *IL6* (Fig 2, *K*; n = 10-27) mRNA expression from PBMCs in asthmatic and healthy children with or without *in vitro* rhinovirus treatment analyzed by using real-time PCR. The Student t test was used to calculate statistical significance. $*P \leq .05$, $**P \leq .01$, and $***P \leq .001$. Results are expressed as means ± SEMs.

T-bet–mediated immune response. At the moment, we do not know which cells are infected by rhinovirus in the PBMC population we examined; however, we assume that plasmacytoid dendritic cells are infected because of the induction of *IDO* after rhinovirus challenge *ex vivo*.[6] However, rhinovirus infection also activates TGF-β present in the environment, as in patients with chronic asthma, to replicate and inhibit effective antiviral immune responses. Thus it is possible that children with acute asthma are able to induce an effective anti-rhinovirus immune response during acute exacerbation. By contrast, in patients with chronic asthma, TGF-β is increased in its active form and is released by structural cells. In this latter situation, when the rhinovirus infects plasmacytoid dendritic cells, this exogenous TGF-β inhibits T_H1 and T_C1 cells that carry the TGF-β receptor, resulting in T_H1 cell depletion, and thus rhinovirus infection cannot be cleared. Although these data need further investigation, they open new avenues for our understanding of the role of rhinovirus-mediated asthma exacerbations in children.

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No evidence of intrauterine sensitization against inhalant allergens

To the Editor:

Allergen-specific IgE can be detected in cord blood (CB), but the origin and relevance of this finding is controversial. We previously reported that specific IgE in CB against inhalant allergens mainly seemed to be the result of transfer of maternal IgE to the fetus,[1] whereas others suggested that such IgE is of fetal origin,[2-6] thus representing intrauterine sensitization. This distinction is important: intrauterine sensitization could be targeted by prenatal intervention and would suggest CB specific IgE as a relevant disease marker in the newborn, whereas maternally transferred IgE, either as a sampling artifact or due to transplacental transfer, would have no independent value as a disease marker and no long-term consequences for allergy in the offspring.

TABLE I. IgA and specific IgE in CB and MB in the present study compared with a previous study*

	Present study N = 260 (%)	Previous study* N = 243 (%)
IgA in CB (>50 μg/L)	3 (1)	21 (9)
Maternal specific IgE >100 kUa/L	4 (2)	12 (5)
Specific IgE in CB, inhalant allergens (kUa/L)	2 (1)	22 (9)
Specific IgE in CB, food allergens (kUa/L)	3 (1)	1 (1)

*Bønnelykke et al.[1]

Here, we aimed to test the hypothesis that specific IgE against inhalant allergens in CB is of maternal origin. We hypothesized that applying strict sampling procedures and including unselected women with low levels of IgE would result in limited, if any, specific IgE in CB.

Women with singleton pregnancies were invited to participate in the study, as previously described.[7] The investigation conformed to the Declaration of Helsinki and was approved by the Scientific Ethics Committee and the Danish Data Surveillance Authority.

Blood was taken from the umbilical vein by needle puncture performed by a single investigator. Samples were available for 260 maternal blood (MB)-CB pairs. IgE antibody levels were determined using the ImmunoCAP assay (Thermo Fisher Scientific, Uppsala, Sweden). MB and CB were analyzed for levels of specific IgE against common inhalant and food allergens (lower detection limit, 0.1 kUa/L) and for total IgE (lower detection limit, 0.1 kU/L using a combination of the standard and low-level total IgE assays). IgA levels in CB samples were analyzed by ELISA (detection limit, 0.1 μg/L). Further information on methodology can be found in this article's Online Repository at www.jacionline.org.

An elevated IgA level in CB (>50 μg/L) was considered indicative of contamination with MB.[1,8] This was found in only 3 (1%) of the CB samples compared with 21 samples (9%) in our previous study.[8] High levels of specific IgE in MB (sum of specific IgE >100 kUa/L) were found in 4 samples (2%) compared with 12 samples (5%) in our previous study[1] (Table I). Correspondingly, specific IgE against inhalant allergens (≥0.1 kUa/L) was found in only 2 CB samples (<1%) compared with 22 (9%) in our previous study[1] (Table I and Fig 1).

In Fig 1, the relationship between specific IgE against inhalant allergens in CB and MB is depicted. We have included prediction lines for the level of specific IgE in CB in relation to the level in MB, stratified by level of CB IgA, based on results from our previous study.[1] As predicted, specific IgE against inhalant allergens in CB was present only when either the level of CB IgA was very high or the corresponding maternal sample had highly elevated levels of specific IgE. For the 2 CB samples with specific IgE against inhalant allergens, corresponding IgE against the same allergen was found in the maternal sample for 2 out of 3 allergens (see Fig E1 in this article's Online Repository at www.jacionline.org).

METHODS

Human study: PreDicta

Our investigations are part of the Europe-wide study PreDicta. The study was approved by the local ethics committee of the Universitätsklinikum Friedrich-Alexander Universität Erlangen-Nürnberg, Germany (Re-No. 4435). Informed consent was obtained from the parents of all participants. The study is registered in the German Clinical Trial Register (Deutsches Register Klinischer Studien: registration no. DRKS00004914). Subject recruitment and some clinical aspects of the children were recently published and described also elsewhere.[E1,E2] This is a longitudinal prospective evaluation over a 2-year period, beginning at the age of 4 to 6 years. In accordance with the PreDicta guidelines, children who met the inclusion criteria in the absence of exclusion criteria were recruited into the study.

Childhood asthma was defined as repeated attacks of airway obstruction and intermittent symptoms of increased airway responsiveness to triggering factors, such as exercise, allergen exposure, and viral infection. The children were asked about shortness of breath (day or night), fatigue, not feeling well, and specific triggers.[E3] Moreover, parents and children were asked to fill diary cards at home 3 times a week on a predetermined time frame. The child's symptom score for the last 24 hours was defined as follows: 0, none; 1, mild, 2, moderate; and 3, severe. When the symptoms were level 4 and higher and there was a decrease in FEV_1 of greater than 15% or peak expiratory flow (PEF) of greater than 30%, the parents were asked to contact the doctor.

Inclusion criteria for the cases were as follows: written informed consent from the child's parent/guardian; age 4 to 6 years; gestational age of 36 weeks or greater; a diagnosis of asthma within the last 2 years confirmed by a doctor of the participating study center; mild-to-moderate persistent severity according to Global Initiative for Asthma 2005 guidelines; 3 episodes in the preceding 12 months; and capacity of the child to perform a PEF maneuver. For examination, the doctor performed lung function measurements, made a clinical investigation (auscultation), and asked the parent/guardian to fill out a questionnaire. Healthy children of the same age with no reported history of asthma/wheeze, atopic illness, or both were recruited at baseline and at the end of the observation period.

Exclusion criteria were severe/brittle asthma; children receiving immunotherapy; more than 6 courses of oral steroids during the preceding 12 months; other chronic respiratory diseases (cystic fibrosis, bronchopulmonary dysplasia, and immunodeficiencies), except allergic rhinitis; and other chronic diseases with continuous medication use, except atopic eczema. Differentiation of asthma bronchiale from bronchiolitis was done mainly based on the clinical diagnosis and additionally mostly based on the presence of rhinovirus in the PCR samples of the nasal swabs. Bronchiolitis in Europe is an inflammatory disease caused by respiratory syncytial virus/rhinovirus infection with tachypnea and diffuse fine crackles, and oxygen is needed in some of these children.

Other wheezing disorders could be anatomically defined, such as stenosis in the airways, hemangioma in the larynx, double aortic arch, and vascular ring/sling. The character of these disorders is continuous and less repetitive, and there are no or few effects of bronchial dilatators.

Study timeline

At the baseline visit (B0) and after 24 months (F4), whole blood was drawn from the children and collected in heparin tubes for PBMC isolation.

If, during the 24-month follow-up period, an asthmatic child experienced an exacerbation, the parents were instructed to call the study center and arrange a visit to the clinic within the next 2 days (symptomatic visit). An exacerbation was defined as follows: a respiratory tract infection/cold; an exacerbation of asthma; a symptom score according to the diary cards of 4 or greater; and a decrease in FEV_1 of greater than 15% or PEF of greater than 30%. At this symptomatic visit, a trained doctor examined and evaluated the child, obtained a nasopharyngeal swab for virus analysis (at the University of Turku), and collected peripheral blood for serum analysis. All exacerbations were analyzed. However, during the entire observation period, only 1 cold per child was analyzed. Cold was defined as symptoms of the upper respiratory tract and general symptoms.

Isolation of PBMCs from children with and without asthma and *in vitro* rhinovirus incubation of PBMCs

At the time of recruitment (B0), PBMCs were isolated from heparinized blood with Ficoll. After isolation, PBMCs were adjusted to a concentration of 1×10^6 viable cells/mL in complete culture medium. For cell culture, RPMI 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% HI-FBS were added (complete culture medium); these reagents were purchased from Sigma-Aldrich (Steinheim, Germany). After PBMC isolation and before cell culture, some PBMCs were infected with RV1B, which was grown, as previously described.[E1]

Rhinovirus infection

For rhinovirus infection, rhinovirus strain RV1B was used. RV1B is currently classified as RV-A species among other 79 rhinovirus serotypes that use intercellular adhesion molecule 1 as their cellular receptor. It is the prototype virus strain and is therefore commonly used in experimental rhinovirus studies.[E4] RV1b was grown, as previously described.[E1] After PBMC isolation and before cell culture, some of the PBMCs were infected with rhinovirus (500 μL/10^6 cells) by shaking for 1 hour at room temperature. After rhinovirus challenge, the cells were washed with medium and cultured in complete culture medium, as were the uninfected cells. Cell culture was performed for 48 hours at 37°C and 5% CO_2. As a control, cells were cultured with cell-culture medium alone. Supernatants were collected for ELISA, and RNA was extracted from the cells for RNA gene arrays and quantitative real-time PCR (qPCR). The HRVA1B titer was tissue culture infectious dose of 50% (TCID50): 10^7.

Quantitative real-time PCR

For human studies, RNA was isolated from PBMCs by using QIAzol (Qiagen, Hilden, Germany), according to the manufacturer's protocol. cDNA synthesis and real-time PCR were performed with the following primers and sequences: human *HPRT* (5′-TGA CAC TGG CAA AAC AAT GCA-3′ and 5′-GGT CCT TTT CAC CAG CAA GCT-3′), human *FOXP3* (5′-AAC AGC ACA TTC CCA GAG TTC CT-3′ and 5′-CAT TGA GTG TCC GCT GCT TCT-3′), human *RORC* (5′-TGA GAA GGA CAG GGA GCC AA-3′ and 5′-CCA CAG ATT TTG CAA GGG ATC A-3′), human *TBX21* (5′-CAG AAT GCC GAG ATT ACT CAG-3′ and 5′-GGT TGG GTA GGA GAG GAG AG-3′), human *TGFB* (5′-CAC GTG GAG CTG TAC CAG AA-3′ and 5′-GAA CCC GTT GAT GTC CAC TT-3′), human *TGFBRII* (5′-TTT TCC ACC TGT GAC AAC CA-3′ and 5′-GGA GAA GCA GCA TCT TCC AG-3′), human *IL6* (5′-TAC CCC CAG GAG AAG ATT CC-3′ and 5′-TTT TCT GCC AGT GCC TCT TT-3′), and human *IDO* (forward, 5′-TGC TGT TCC TTA CTG CCA AC-3′; reverse, 5′- CGT CCA TGT TCT CAT AAG TCA GG-3′).

Gene arrays

For RNA gene arrays, only RNA that passed high-stringency quality controls was reverse transcribed. Biotinylated cDNA was prepared according to the standard Illumina protocol (Illumina, San Diego, Calif) from 13.9 ng of total RNA by using the amplification kit Pico WTA (lot no. 1404311-A/C), followed by labeling 3 μg of cDNA with the kit Encore BiotinIL module (lot no. 1212262-D). BeadChips were scanned by using the Illumina iScan Scanner running Illumina iSCAN control software, version 3.3.28. Data were analyzed with Illumina GenomeStudio, version 2011. Heat maps were generated by performing supervised clustering on normalized expression data with R software, version 3.1, and library gplots. Genes selected for analysis showed statistically significant regulation by rhinovirus.[E5]

ELISA

Human TGF-β1 was detected in cell-culture supernatants by using OptEIA sandwich ELISA kits from BD Biosciences (Heidelberg, Germany), according to the manufacturer's protocol.

Mice

Wild-type mice were on a BALB/c genetic background. The experiments were performed with age- and sex-matched mice at the age of 6 to 8 weeks. The animals were bred in the animal facility adjacent to our institute with temperature control and had free access to food and water. All experiments were performed with approved licenses (23-177-07/G09-1-008 from the ethical review board Rheinland-Pfalz and 54-2532.1-55/12 from the government of Lower Franconia, Germany).

Experimental allergic asthma and total lung cell culture

Wild-type BALB/c mice were treated at day 0 of the ovalbumin (OVA) protocol with a 200-μL injection of OVA complexed with alum (100 μg) intraperitoneally (500 μg/mL). At days 7, 8, and 9, 25 μL of OVA dissolved in PBS was administered (2 mg/mL). At day 10, mice were killed, and lung cells were isolated, as previously described.[E6] Isolated cells were cultured at a density of 1 million cells per milliliter in RPMI 1640 medium supplemented with 10% FCS, 1% L-glutamine, and 1% penicillin and streptomycin, respectively. Cells were either left untreated or incubated with rhinovirus (see below). Cell culture was performed in the presence of absence of 10 ng/mL TGF-β for 48 hours at 37°C and 5% CO_2. After culturing, supernatants were collected and cells were used for flow cytometry or RNA isolation. Experimental setups are depicted in Figs E2, *A*, and E3, *A*.

In vitro rhinovirus treatment of murine total lung cells

For rhinovirus infection, total lung cells were incubated with rhinovirus (500 μL of rhinovirus/10^6 cells) on a horizontal shaker for 1 hour at room temperature, as described for PBMC cultures above.

RNA isolation and quantitative real-time PCR in murine total lung cells

RNA was extracted from murine total lung cells by using PeqGold RNA Pure, according to the manufacturer's protocol (PeqLab, Erlangen, Germany). Cells were directly resuspended in PeqGold RNA Pure for RNA isolation. RNA (1 μg) was then reverse transcribed by using the first-strand cDNA synthesis kit for RT-PCR (MBI Fermentas, St-Leon-Rot, Germany). The resulting template cDNA was amplified by means of qPCR with SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Munich, Germany). qPCR was performed with a cycle of 2 minutes at 98°C and 50 cycles of 5 seconds at 95°C and 10 seconds at 60°C, followed by 5 seconds at 65°C and 5 seconds at 95°C in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories). The primers and sequences used for mice were as follows: mouse *Ido* (5′-GGC CCA TGA CAT ACG AGA ACA-3′ and 5′-AGA AGC TGC GAT TTC CAC CA-3′). The mRNA of the genes of interest was normalized by using the mRNA levels of the housekeeping gene mouse *Hprt* (5′-GCC CCA AAA TGG TTA AGG TT-3′ and 5′-TTG CGC TCA TCT TAG GCT TT-3′).

Flow cytometric analysis and intracellular staining

Total lung cells were cultured as indicated above. After 48 hours, cells were harvested, washed, and stained with anti-CD4 or anti-CD8 antibodies (BD Biosciences) for 30 minutes at 4°C, and for intracellular staining, the cells were washed and permeabilized with Permwash and Permfix (BD Biosciences) for 35 minutes at 4°C. After washing, cells were stained in stain buffer (BD Biosciences) with anti–T-bet or anti–IFN-γ antibodies (BD Biosciences) for 30 minutes at 4°C. Cells were washed and resolved in stain buffer, acquired by using a FACSCalibur (BD Biosciences), and analyzed with FlowJo software (TreeStar, Ashland, Ore).

For intracellular cytokine staining, cells were stimulated with phorbol 12-myristate 13-acetate (50 ng/mL), ionomycin (500 ng/mL), and protein transport inhibitor (GolgiStop, BD Biosciences) for 4 hours in accordance with the manufacturer's instructions and as previously described.

Rhinovirus PCR in human PBMCs

PCR was performed to verify that infection with RV1b in PBMCs was successful, as described below. RNA was isolated with PeqGold RNA pure and reverse transcribed into cDNA. Subsequently, 2 μL of cDNA, 0.5 μL per primer (OL26: 5′-GCA CTT CTG TTT CCC C-3′; OL27: CGG ACA CCC AAA GTA G), 4.5 μL of diethyl pyrocarbonate H_2O, and 12.5 μL of KAPA2G Fast Ready Mix were used. The rhinovirus PCR runs were in 32 cycles, starting with denaturation (at 94°C for 30 seconds), followed by primer annealing (at 50°C for 30 seconds) and elongation (at 72°C for 2 minutes), completed by a post-PCR extending step (at 72°C for 4 minutes). Therefore an amplicon of 380 bp was generated, which was analyzed and quantified with QIAxcel Advanced Systems (Qiagen).

Statistical analysis

Differences were evaluated for significance by using the Student *t* test. Data are presented as mean values ± SEMs.

RESULTS

The average age of control subjects and cases was 4.7 and 4.9 years, respectively. By rating the severity of the disease according to Global Initiative for Asthma guidelines (2005), 60% of the children had intermittent, 25% had mild persistent, and 10% had moderate persistent asthma. Steroid or steroid/nonsteroid drug combined treatment was used by 79% of children with asthma. Analysis of the lung function of cases and control subjects showed that 72.7% of control subjects and only 45.8% of the children with asthma had an FEV_1 of greater than 100% (see Table E1).

We also investigated which T-cell type is selected after *ex vivo* infection of lung cells from naive and asthmatic mice with rhinovirus (Fig E2, *A*). Consistent with human data, we observed that in naive lung cells rhinovirus induces an effective antiviral immune response by upregulating T_C1 cells ($CD8^+IFN\text{-}\gamma^+$; Fig E2, *B*).

It is known that rhinovirus is able to directly activate T cells.[E7] To determine whether exogenous active TGF-β would influence the T-cell immune response to rhinovirus, we exposed lung cells from naive and asthmatic wild-type mice with rhinovirus *in vitro* and then cultured them with TGF-β (see Fig E3, *A*). In naive total lung cells TGF-β inhibited *Ido* expression in rhinovirus-treated cells (Fig E3, *B*). Here we found that, consistent with an immunosuppressive role of TGF-β on T_H1 cells,[E8] $CD4^+$T-bet^+ cells were inhibited (Fig E3, *B*).

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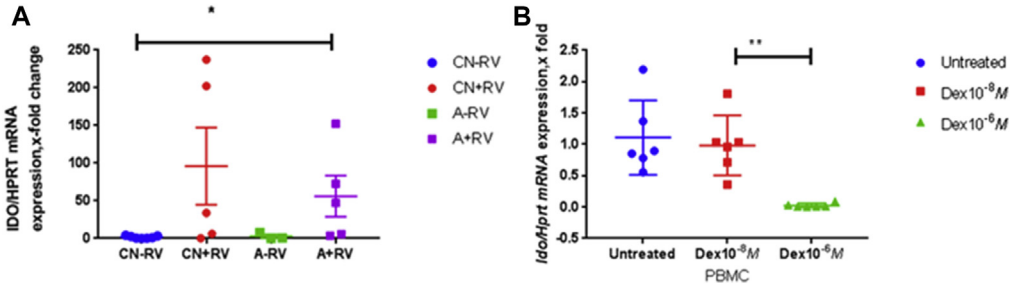

FIG E1. MRNA expression of *IDO* in PBMCs. **A,** *IDO* mRNA expression in PBMCs from asthmatic *(A)* and nonasthmatic *(CN)* children with *(+RV)* or without *(−RV) in vitro* rhinovirus treatment. **B,** PBMCs from healthy volunteers were *in vitro* incubated with different concentrations of dexamethasone *(Dex)*, 10^{-6} mol/L and 10^{-8} mol/L, and *IDO* expression was determined by using qPCR. $*P \leq .05$ and $**P \leq .01$.

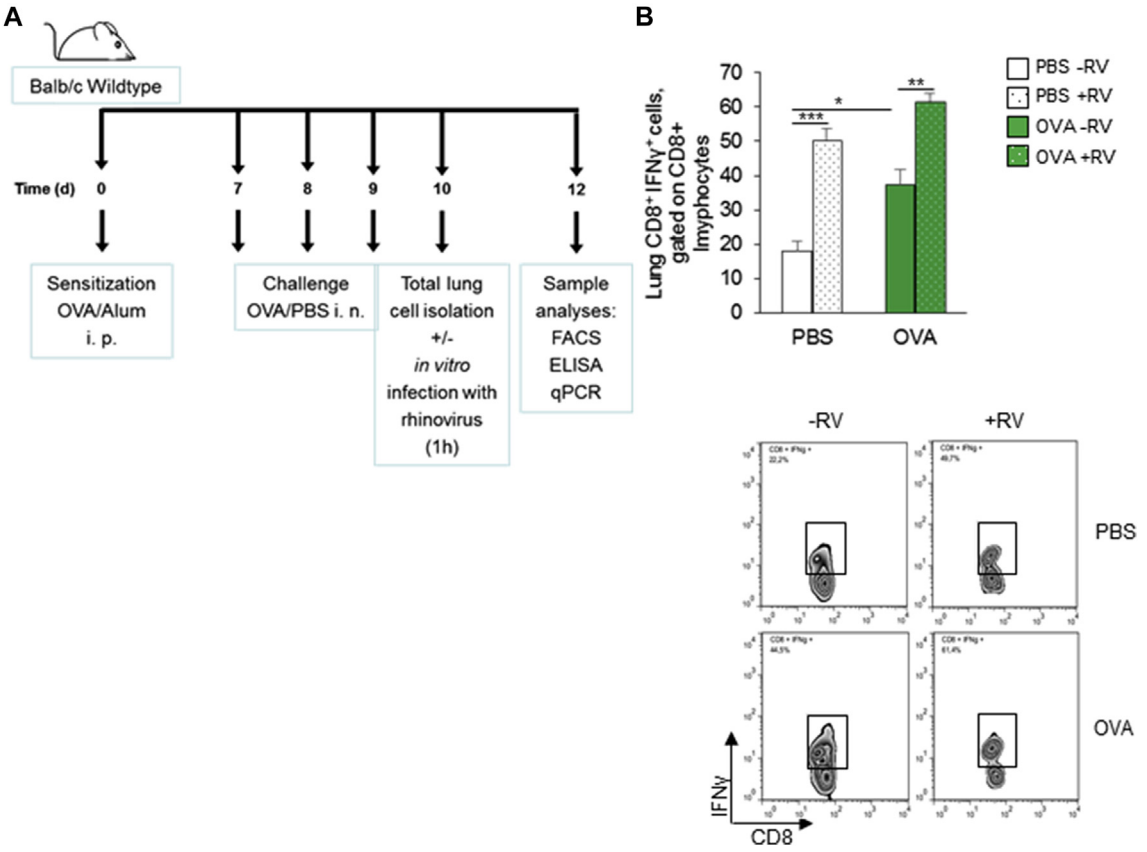

FIG E2. Rhinovirus induces T_C1 cells in a murine model of asthma. **A,** Experimental design. *i.n.*, Intranasal; *i.p.*, intraperitoneal. **B,** $CD8^+IFN\text{-}\gamma^+$ cells were analyzed in total lung cells by using flow cytometry. A dot plot is shown for each group (n = 4-5 mice per group). $*P \leq .05$, $**P \leq .01$, and $***P \leq .001$.

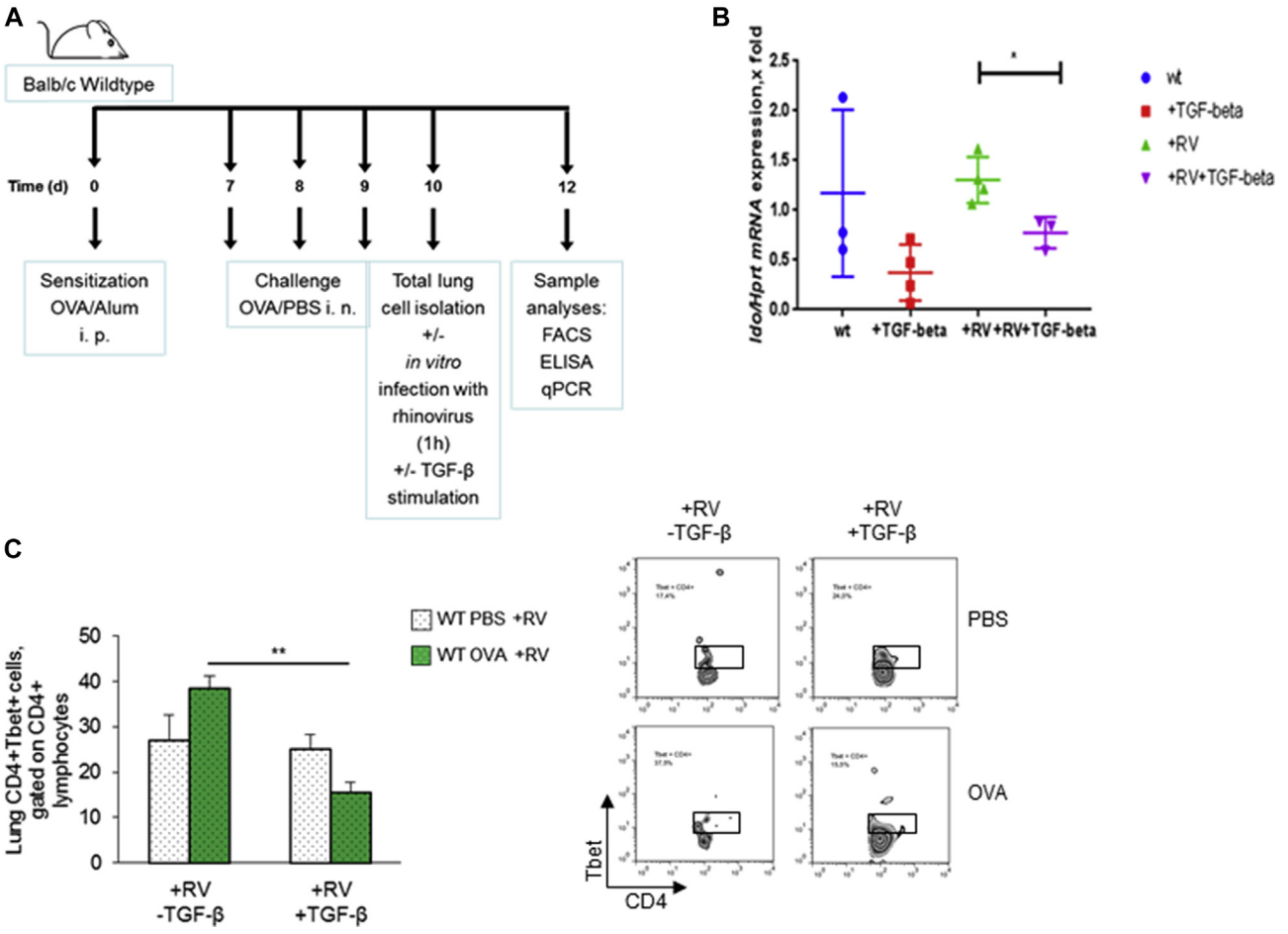

FIG E3. TGF-β treatment of rhinovirus-infected cells reduces T-bet expression in $CD4^+$ T cells in a murine model of asthma. **A,** Experimental design. *i.n.*, Intranasal; *i.p.*, intraperitoneal. **B,** *Ido* mRNA expression was detected in total lung cells from naive mice cultured with TGF-β after *in vitro* treatment with rhinovirus or untreated (n = 4). **C,** $CD4^+$T-bet^+ cells were analyzed in total lung cells from naive or OVA-treated mice by using flow cytometry. Exemplary dot plots are depicted for each group analyzed (n = 4-5 mice per group). $^*P \leq .05$ and $^{**}P \leq .01$.

TABLE E1. Demographic and clinical data of the PreDicta cohorts WP1-UK-ER analyzed

Asthmatic patients								Control subjects			
	Age (y)	Sex	Phenotype	Treatment	Asthma	Skin prick test	FEV_1 (%)		Age (y)	Sex	FEV_1 (%)
201	6	M	V	s + n (S+LTRA)	c	al, ca, g	126	208	6	M	77
202	6	M	U	st	p	al, b, g	80	211	6	F	121
203	5	F	U	st + ah	p	ca	101	214	5	M	112
204	6	M	A	st + n (S)	c	al, am, ca, g, h	128	215	4	M	—
205	5	M	U	st + n (S)	p	/	102	218	4	F	118
206	5	F	U	st	c	al	129	219	5	F	111
207	5	M	V	st + n (S)	p	g	143	220	5	F	96
209	4	F	A, V	st	p	g	115	221	3	M	81
210	6	F	V	n (S)	p	b, g	94	222	6	M	105
212	5	M	E, V	st + n (S)	p	—	98	226	4	F	109
213	4	M	E	st + n (S)	p	—	115	227	6	M	87
216	5	F	A, V	st + n (S)	u	ca, g, h	92	232	4	M	100
217	6	F	A, E, V	st	c	b, g, h	111	233	5	F	112
223	5	M	V	st + n (LTRA)	c	ca, f, g, h	99	234	5	F	119
224	4	F	V	st	c	—	135	235	4	M	116
225	4	M	V	st + n (S)	c	/	97	236	5	M	111
228	5	M	V	—	c	ca, g, h	84	237	4	M	109
229	4	M	V	n (S)	c	al, b, ca, g, h	86	240	4	F	92
230	5	M	V	—	c	al, am, b, ca, g, h	107	241	5	M	123
231	4	M	V	st + n (S)	c	b	71	244	5	F	107
238	4	M	V	st + n (S)	c	am, ca, g, h	86	245	4	M	121
239	5	F	E	n (ah)	c	/	98	246		M	109
242	5	M	A, E, V	st + n (ah)	c	/	81				
243	5	F	V	st + n (S)	u	al, am, h	69				
Average	4.9 ± 0.1						101.9 ± 4.0		4.7 ±0.18		105.7 ± 3.0

A, Allergen induced; *ah*, antihistamine; *al*, *Alternaria* species; *am*, ambrosia; *b*, birch; *ca*, cat; *c*, controlled; *E*, exercise-induced; *F*, female; *f*, *Dermatophagoides farinae*; *g*, grass pollen mix; *h*, house dust mite; *LTRA*, leukotriene antagonist; *M*, male; *n*, nonsteroid treatment; *p*, partially controlled; *st+S*, steroid treatment; *U*, unresolved; *u*, uncontrolled; *V*, virus-induced asthma.

TABLE E2. Viral colonization in nasal pharyngeal fluid (NPF) at baseline (B0)

Asthmatic patients	Rhinovirus	Control subjects	Rhinovirus
201	++	208	−
202	++	211	++
203	−	214	++
204	−	215	−
205	−	218	+++
206	++	219	+
207	−	220	−
209	−	221	++
210	CoVNL63++	222	−
212	Flu A++	226	+
213	++	227	+
216	−	232	+++
217	−	233	+, MPV+
223	+++	234	++, AdV++
224	++, PIV4+	235	++
225	++, PIV4+	236	−
228	−	240	+, PIV4+, HBoV+, FluA++
229	+++	241	++
230	+	245	−
231	HBoV++, CoVNL63+++	246	+
238	+++		
239	+		
242	+, HBoV+, RSVB++, HEV++, PIRV++, PIV4, AdV+		
243	+++		

In some cases other respiratory viruses were detected as follows: *AdV*, Adenovirus; *CoV (NL63)*, human coronavirus (NL63); *FluA/B*, influenza virus A/B; *HBoV*, human bocavirus; *HEV*, enterovirus; *MPV*, human metapneumovirus; *OC43/HK1*, human coronavirus (OC43/HK1); *PIV2/4*, parainfluenza virus 1-4; *RSVA/B*, respiratory syncytial virus A/B; *RV*, rhinovirus.

+, Low copy number of rhinovirus; ++, intermediate copy number of rhinovirus; +++, high copy number of viral genomes detected in the sample; −, negative, no virus detected.

TABLE E3. Viral detection in nasal pharyngeal fluid (NPF) at the end point visit and 24 months (F4) after baseline (B0) in asthmatic and control children of the Erlangen cohort analyzed in gene arrays

Control subjects (n = 6)	F4	Asthmatic patients (n = 8)	F4
215	RV+, AdV+++	202	RV++
220	−	204	RV
221	−	205	RV+++
222	−	210	HEV
226	RV++	212	RV+
227	−	216	RV++
		217	RV++
		223	No sample

+, Low copy number; ++, intermediate copy number; +++, high copy number of viral genomes detected in the sample; −, negative, no virus detected; *AdV*, adenovirus; *HEV*, human enterovirus; *RV*, rhinovirus.

TABLE E4. Medications taken by the asthmatic children analyzed in gene arrays

Asthma F4	Rhinovirus *in vitro*	Medication
202	− and +	Steroid
204	− and +	Steroid + β_2-agonists
205	− and +	Steroid + β_2-agonist
212	− and +	Steroid + β_2-agonists
217	− and +	Steroids
216	Only −	Steroids + β_2-agonists
223	Only +	Steroid + LTRA

LTRA, Leukotriene antagonist.

TABLE E5. Meaning of the genes shown in the heat maps

CTLA4	Expressed by activated T cells. Other T cells can receive an inhibitory signal from CTLA4 to prevent an overreaction of the immune system.
IDO1	Tryptophan-converting enzyme that promotes T cell–mediated tolerance and antimicrobial effects. Several diseases are associated with increased expression.
CD274	Encodes an immune inhibitory receptor ligand. Can inhibit T-cell activation and cytokine production, which is essential for preventing autoimmunity (PD-L1).
IL1B	Encodes a protein that is a member of the IL-1 cytokine family. This cytokine is an important mediator of the inflammatory response and is involved in cell proliferation, differentiation, and apoptosis.
IFITM2	Encodes an interferon-induced antiviral protein that inhibits entry of viruses to the host cell cytoplasm, permitting endocytosis but preventing subsequent viral fusion and release of viral contents into the cytosol.
LDLR	Encodes a protein called a low-density lipoprotein receptor. This receptor binds to low-density lipoproteins, which are the primary carriers of cholesterol in the blood. Also, some rhinoviruses bind to the LDL receptor.
TLR8	Encodes a gene that is a member of the Toll-like receptor family. Takes part in pathogen recognition and activation of innate immunity.
MAD2L2	MAD2L2 is a component of the mitotic spindle assembly checkpoint that prevents the onset of anaphase until all chromosomes are properly aligned at the metaphase plate.
FAS	Encodes a protein belonging to the TNF receptor superfamily. It contains a death domain and has been shown to play a central role in the physiologic regulation of programmed cell death.
CDY2B	Encodes a protein containing a chromodomain and a histone acetyltransferase catalytic domain. Chromodomain proteins are components of heterochromatin-like complexes and can act as gene repressors.
TGIF2	Transcriptional repressor that can repress transcription by recruiting histone deacetylases to TGF-β–responsive genes.
RNASE1	Encodes a member of the pancreatic type of secretory ribonucleases, which cleave internal phosphodiester RNA bonds on the 3′ side of pyrimidine bases.
EVI5	An oncogene that can regulate the stability and accumulation of critical G_1 cell-cycle factors, including Emi1 and cyclin A.
RHOG	Belongs to the family of G proteins and is involved in cellular signaling mechanisms, cytoskeletal reorganization, and subsequent morphologic changes in various cell types.
LY6E	Induced by IFN-α and is associated with drug resistance and tumor immune escape.
IL32	A proinflammatory cytokine that can induce cells of the immune system to secrete inflammatory cytokines, such as TNF-α and IL-6.
RNASE6	Belongs to the RNase A superfamily. Its expression is induced in neutrophils and monocytes on bacterial infection, suggesting a role in host defense.