Increased antiviral response in circulating lymphocytes from hypogammaglobulinemia patients.

Oliver F Wirz[1 #], Öykü Üzülmez[2], Kirstin Jansen[1], Willem van de Veen[1,3], Auli Lammela[4], Leena Kainulainen[4], Tytti Vuorinen[5], Heimo Breiteneder[2], Cezmi A Akdis[1,3], Tuomas Jartti[4] and Mübeccel Akdis[1] *

1 Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland.
2 Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria.
3 Christine Kühne – Center for Allergy Research and Education (CK-CARE), Davos, Switzerland.
4 Department of Pediatrics and Adolescent Medicine, Turku University Hospital and University of Turku, Turku, Finland.
5 Department of Clinical Virology, Turku University Hospital and Department of Virology, University of Turku, Turku, Finland.

Current address: Department of Pathology, Stanford University, Stanford, CA 94305, USA.

* Corresponding author:
Mübeccel Akdis, MD, PhD.
Swiss Institute of Allergy and Asthma Research (SIAF)
Herman-Burchard-Strasse 9
CH-7265 Davos-Wolfgang, Switzerland
E-mail: akdism@siaf.uzh.ch
Tel.: +41 81 410 08 48
Fax: +41 81 410 08 40

Short title

Antiviral response in hypogammaglobulinemia patients.

Abbreviations

BCR B cell receptor

CD	Cluster of differentiation
HG	Hypogammaglobulinemia
RV	Rhinovirus
IFI44L	Gene encoding Interferon Induced Protein 44 Like
IFIT1	Gene encoding Interferon-induced protein with tetratricopeptide repeats 1
IFITM1	Gene encoding Interferon-induced transmembrane protein 1
IFN	Interferon
Ig	Immunoglobulin
MX1	Gene encoding Interferon-induced GTP-binding protein Mx1
PBMC	Peripheral blood mononuclear cells
RNA	Ribonucleic acid
RT-PCR	Real-time polymerase chain-reaction
RV	Rhinovirus
STAT1	Gene encoding Signal transducer and activator of transcription 1

Word count

3262 (main text without abstract), 296 (abstract).

Acknowledgement

We thank all the patients and their physicians for their participation. We thank laborant Heidi Jokinen for PBMC sample processing and clinical virus diagnostics. M. Akdis has received research support from the Swiss National Science Foundation No. 320030-159870/310030-179428 and the European Commission's Seventh Framework Programme PREDICTA (No: 260895) and the Sean N Parker Center for Allergy and Asthma Research at Stanford University. C.A. Akdis is employed by the Swiss Institute of Allergy and Asthma Research, University of Zurich;, the Swiss National Science Foundation No. 310030-156823, and the Christine Kühne – Center for Allergy Research and Education (CK-CARE). Tuomas Jartti has recieved research support from Sigrid Juselius Foundation, Paulo Foundation and Finnish Society for Allergy and Immunology, all in Helsinki, Finland. Authors H. Breiteneder & Ö. Üzülmez acknowledge the support of the Austrian Science Fund (FWF) Doctoral Program MCCA W1248-B30.

Abstract

Background: Rhinovirus (RV) is the main cause of respiratory tract infections. B cells play a crucial role during these infections by production of virus neutralizing antibodies. Patients with hypogammaglubolinemia (HG) often have severely reduced levels of antibody producing B cells and suffer from prolonged virus infection. Here, we addressed whether antiviral response of B cells, T cells and monocytes from peripheral blood differs between in HG patients and healthy individuals during natural RV infection.

Methods: Using fluorescence-activated cell sorting, CD19+ B cells, CD14+ monocytes and CD3+ T cells were isolated from frozen peripheral blood mononuclear cells (PBMC) from 11 RV-infected hypogammaglobulinemia patients, 7 RV-infected control subjects and 14 non-infected control subjects. Flow cytometry was used for detection of different B cell subsets and real-time PCR to study expression of antiviral genes. A pan-RV PCR was used to detect RV genome in all samples.

Results: In HG patients, total B cell numbers, as well as IgA- and IgG-switched memory B cells were reduced with RV infection. The number of naïve B cells was increased. A dominant T cell response with significantly increased numbers of CD3+ cells was observed in RV-infected HG patients, whereas the numbers of monocytes did not change. The expression of *STAT1* was increased in HG patients compared to controls in all three lymphocyte subsets. The expression of antiviral genes *IFITM1* and *MX1* correlated with *STAT1* expression in B cells and monocytes. RV RNA was found in 88.9 % of monocytes of infected HG patients, 85.7 % of monocytes of infected controls without HG and 7.1 % of monocytes of uninfected controls.

Conclusions: We demonstrate an increased antiviral response in B cells and monocytes in HG patients and their correlation with STAT1 expression. Monocytes of infected HG patients and infected non-HG controls carry RV RNA.

Keywords

Hypogammaglobulinemia, CVID, Rhinovirus, Lymphocytes, B cells, Monocytes, Antiviral response.

Introduction

Around 50-70% of upper respiratory tract infections are caused by rhinovirus (RV), making them the most common cause of viral induced respiratory diseases [1,2]. While RV infections are usually not life-threatening for healthy individuals, RV-induced bronchiolitis in early life was linked to increased risk for development of asthma [3-6]. In addition, RV infections are responsible for the majority of detrimental asthma exacerbations [7]. Neutralizing antibodies play a crucial role during virus infection and protect against reinfection with the same RV strain [8,9]. In patients with hypogammaglobulinemia (HG), the frequencies of circulating B cells, IgG+ and IgA+ memory B cells, which typically show a higher level of affinity maturation are usually reduced [10]. Decreased levels of virus neutralizing antibodies lead to recurrent and severe rhinovirus infections [11]. Furthermore, the time of virus shedding is markedly prolonged in rhinovirus infected patients with HG [12].

Rhinovirus infection in humans induces type-I interferon (IFN) secretion which leads to expression of interferon stimulated genes (ISG) in cells of peripheral blood [13]. Increased virus levels as they were found in mucosal tissues of HG patients [12] are likely to stimulate a more pronounced antiviral response in epithelial cells and phagocytes of the airways. Indeed, in patients where common variable immune deficiency (CVID) is characterized by reduced levels of antibody-producing B cells, a marked increase in IFN-signalling was observed in full blood RNA-sequencing [14]. In contrast, increased expression of ISGs was not observed in CVID without lymphopenia [14]. After virus exposure, a type-I IFN response is initiated by infected cells. Upon binding of type-I IFNs to their cellular receptor, the transcription factor STAT1 is expressed and phosphorylated [15,16]. STAT1 then induces the expression of many downstream ISGs, including the chemokine CXCL10 (IP-10) and antiviral genes such as IFITM1 and MX1, that interfere with different stages of the viral life cycle [17].

Prior studies focusing on total PBMC showed elevated expression of ISGs at steady state condition in HG patients.[14,18] The aim of this study was to address the antiviral response in different leukocytes in HG patients. For this we used a well-characterized cohort of HG patients who were naturally infected with rhinovirus as well as a naturally infected and non-infected control groups without any other disease. Using flow cytometric analysis, we analysed the B cell compartment in detail demonstrating reduced total and switched IgA+ and IgG+ memory B cells. Then, we analysed RNA expression of antiviral response genes in CD19+ B cells, CD14+ monocytes and CD3+ T cells that were sorted from blood of infected donors during episodes of active RV infection. Overall, we demonstrate an increased antiviral response in different circulating immune cell types in hypogammaglobulinemia patients and we show correlation of virus load on expression of antiviral genes in peripheral monocytes.

Methods

Study Design

HG patients were recruited from the Immunodeficiency out-patient Clinic of Turku University Hospital. Patients visited the clinic for regular follow-up and for immunoglobulin replacement therapy. Healthy control subjects were recruited from hospital staff and came for unscheduled visit when they had common cold symptoms. Non-infected control subjects served as negative control group. The study protocol was approved by the Ethics Committee of the Turku University Hospital and commenced only after obtaining written informed consent from the patient.

Purification of PBMC and lymphocyte subsets, flow cytometry and sample preparation

Peripheral blood mononuclear cells (PBMC) from naturally infected individuals and healthy subjects were defrosted and washed with complete RPMI 1640 medium supplemented with L-glutamine (2 mmol/l), MEM vitamin, penicillin, streptomycin, kanamycin, nonessential amino acids, sodium pyruvate (Life Technologies, Carlsbad, CA, USA), and 10% heat-inactivated fetal calf serum (FCS; Invitrogen, Carlsbad, CA, USA). Then, 300'000 PBMC were directly frozen for later RNA extraction. The rest of the PBMC were resuspended in staining buffer (PBS, 0.5% BSA) and stained with antibodies against CD19, CD3, CD14, CD24, CD27, kappa light chain, CD38, IgD, IgM, IgA, CD10, IgG, lambda light chain and viability dye for flow cytometry (**Table S3**). Cells were incubated for 30 min at 4°C, washed and filtered prior to sorting. Then, CD19+, CD14+ and CD3+ cells were sorted using a BD ARIA IIIu (Becton Dickinson, Franklin Lanes, NJ) (**Fig S1**). Sorted cells were lysed immediately using RLT plus buffer (QIAGEN, Hilden, Germany). Cell lysates were stored at -80°C until total RNA was isolated using RNeasy Plus Micro Kit (QIAGEN). RNA was stored at -80°C until it was analysed.

Real-time PCR

cDNA was synthesized with RevertAid RT Reverse Transcription KIT (Thermo Fisher Scientific) according to manufacturer's protocol. cDNAs were amplified with Maxima SYBR Green/ROX qPCR Master Mix (2X), (Thermo Fisher Scientific) and 0.6 µM of primers (**Table S4**). Thermal cycling was performed with a two-step cycling protocol according to the manufacturer's recommendations using a Quant Studio 7 Flex (Thermo Fisher Scientific). Gene expression was normalized with the housekeeping gene EF1α.

Rhinovirus detection

For clinical diagnostics, in-house real-time PCR assay was used to detect RV as described previously [19]. The diagnostics was carried out in the Department of Virology, University of Turku, Turku, Finland. For PBMC, high sensitivity detection of RV was performed using a previously described PCR protocol [20]. In short, RNA was isolated from peripheral cells subsets, transcribed to cDNA and used for the viral detection two-step PCRs. Pan-RV primers were used for the PCR: a mix of three forward primers *B1*

5'-CAA GCA CTT CTG TTT CCC C-3', *B2 5'-CAA GCA CTT CTG TTA CCC C-3'* and *B3 5'-CAA GCA CTT CTG TCT CCC C-3'* (25µM each) and reverse *FR2 5'-ACG GAC ACC CAA AGT AG-3'* (25µM)[20]. As a positive control, RV-29 was used. Resulting bands have approximate size of 400 bp. Relative band intensities were measured using *Evolution-Capt Edge*-software from Vilber Lourmat (France) using standard settings, including background subtraction "Rolling ball" (ball size of 14). The positive control of each gel was set to 100 % and sample band intensities were compared to this positive control.

Statistics

Differences between groups were analysed using two-sample t-test for normally distributed and Wilcoxon rank-sum test for non-normally distributed continuous variables. Categorical variables were analysed using Chi-square test, or Fischer's exact test (when counts less than 5). In all statistical analyses, two-sided tests were used. We used a significance level of 0.05. We used GraphPad Prism version 8.1.1 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com).

Results

Study cohort and subject characteristics

PBMC were isolated from a total of 11 RV-infected HG patients at 18 illness episodes, 7 RV-infected control subjects and 14 uninfected control subjects. HG group consisted of 9 common variable immunodeficiency (CVID) and 2 X-linked agammaglobulinemia (XLA) patients (**Tables S1 and S2**). Mean age of HG patients was 55 years (SD 10) and 63% were males. Control subjects differed from the patients by age (mean 27 years, SD 14) and sex (28% were males) (for both t test $p<0.05$). Duration of symptoms was difficult to estimate in HG patients due to their chronicity. However, the estimated duration of illnesses was also longer in HG patients compared to controls: medians (interquartile range), 4 (2, 11) vs. 1 (1, 2) ($p<0.05$).

Hypogammaglobulinemia patients show reduced total and memory B cells.

We first addressed whether the numbers of cell subsets we planned to study differed among the study groups. For this, PBMC from naturally infected HG patients, infected control non-HG subjects and noninfected healthy control subjects were defrosted and subjected to flow cytometric analysis (**Fig S2**). We first focused on different B cell subsets, as HG patients suffer from inefficient B cell responses. As was reported in previous studies [21,22], the level of total B cells did not change upon rhinovirus infection in healthy controls. While total B cell levels were decreased in infected HG patients compared to infected healthy (**Fig 1A**), the numbers of non-switched IgM+ IgD+ B cells (**Fig 1B**), immature CD10+ B cells (**Fig 1C**) and transitional B cells (**Fig 1D**) was similar between the groups. In contrast to these less mature B cell subsets, switched IgD+ and IgM+ B cells (**Fig 1E** and **F**) were increased in infected HG patients. In line with previous findings, number and percentage of switched IgM- IgD- memory B cells (**Fig 1G**), as well as IgA+ and IgG+ memory B cells (**Fig 1H** and **I**) were strongly decreased in HG patients. When looking at switched IgM- IgD- B cells in the three patient groups, a clear shift in isotype usage was found with almost doubling of switched IgM+ and IgD+ B cell fraction (**Fig 1J**). No difference was found between the groups in the levels of kappa- and lambda-light chain expression in most of the individual B cell subsets (**Fig S3**). However, while the kappa/lambda-ratio was very similar in control groups, the ratio was distributed on a larger range in HG patients indicating possible dysregulation. In addition to B cells, we found that the number of monocytes was increased in infected controls compared to uninfected (**Fig 1K**) while the number of CD3+ T cells from total lymphocytes was increased in infected HG patients compared to infected control group, demonstrating a T cell-dominant response in B cell-deficient patients. These data demonstrate that HG patients respond to RV infection with a decreased capacity to switch to memory B cell response with particularly reduced IgA and IgG positive memory B cell numbers. B cells stay in naïve stage and the number of T cells increase.

Expression of RV-response related genes in PBMC and different blood cell subsets.

Next, we wanted to address whether the response to RV infection is altered in HG patients in the different cell subsets of the blood. Therefore, we sorted CD19+ B cells, CD14+ monocytes and CD3+ T cells from PBMC (**Fig S1**) and analysed RNA expression of essential antiviral genes in the different cell subsets. Firstly, we examined RNA expression of *STAT1* because virus-induced type-I IFNs signal via this transcription factor to induce antiviral response genes and upregulates its expression [16]. Furthermore, we analysed the expression of antiviral response genes that we previously found to be induced in peripheral blood B cells after experimental rhinovirus infection (data not published). As expected, *STAT1* was upregulated in infected control compared to non-infected healthy controls (**Fig 2A**). Moreover, genes further downstream of *STAT1*, coding for functional proteins *IFITM1*, *MX1*, *IFIT1*, and IFI44L were also upregulated upon infection in PBMC (**Fig 2A** and **Fig S4**). Interestingly, in HG patients, expression of *STAT1* is increased in B cells, monocytes and T cells compared to infected control (**Fig 2B**-**D**). While this was not shown for different cell subsets of the blood before, these results are in line with earlier findings showing increased *STAT1* expression in some CVID patients [23,24]. Furthermore, expression of *IFITM1* in B and T cells and *MX1* in B cells seems slightly increased in HG patients compared to control. Overall, we observed a slight increase in antiviral genes expression upon infection in PBMC, B cells, monocytes, and T cells and increased expression of *STAT1* in HG patients.

Expression of antiviral genes correlates with transcription factor STAT1-expression.

Since the expression of antiviral genes was only slightly increased in HG patients, we addressed, whether their expression correlated with the expression of the transcription factor *STAT1*, which is responsible for their induction. We found that *IFITM1* in B cells and T cells correlated with *STAT1* expression with a similar tendency in monocytes (**Fig 3A**). Furthermore, *MX1* expression correlated with *STAT1* expression in B cells and monocytes (**Fig 3B**). For PBMC and monocytes, we also assessed expression of *CXCL10* (IP-10), a chemokine which is induced by type-I and type-II IFNs via transcription factor STAT1 or upon rhinovirus-infection in monocytes [25,26]. *CXCL10* expression was slightly (but not significantly) increased in monocytes from infected HG patients compared to infected controls (**Fig 3C**). Interestingly, expression of this chemokine strongly correlated with *STAT1* expression in PBMC, as well as in monocytes (**Fig 3D**). These data suggest a possible link between increased STAT1 expression found in HG patients (**Fig 2A** and **C**) and expression of the chemokine CXCL10. Overall, we show that *STAT1*-expression correlates with expression of antiviral genes known to be induced by STAT1.

Monocytes carry rhinovirus RNA after natural infection with rhinovirus.

We found increased antiviral gene expression in different peripheral blood cell subsets. This may result from stimulation with type-I-interferons when trafficking through infected airway tissue. Another possibility is that these cells were directly infected with RV when trafficking infected tissue. To test this, we performed a pan-RV PCR on cDNA from PBMC, CD19+ B cells, CD3+ T cells and CD14+ monocytes. From all subsets tested, only CD14+ monocytes showed positive bands of varying intensity. As expected, the non-infected control group was largely negative (**Fig 4A**), while 6 out of 7 samples were positive in the infected control group (**Fig 4B**) and 16/18 samples were positive in infected HG patients (**Fig 4C**). Overall, 85.7 to 88.9% of infected subjects had monocytes carrying RV-derived RNA (**Fig 4D**).

Increased expression of antiviral gene STAT1 and chemokine CXCL10 correlates with higher viral load in circulating monocytes.

Next, we wanted to assess whether the expression of antiviral genes in monocytes depends on viral load. For this, band intensities were calculated and compared to positive control. This way we can compare band intensities among samples of different gels. Samples were grouped into negative/low (≤45 % of positive control), intermediate (45 to 90 % of positive control), and high (≥ 90% of positive control)**.** Interestingly, expression of the gene encoding for transcription factor STAT1 was increased in samples with high virus load (**Fig 5A**). Furthermore, expression of chemokine gene *CXCL10* was upregulated in samples with intermediate and high virus load when compared to the group that was low/negative for virus load (**Fig 5B**). This suggests that monocytes not only take up RV but also seem to respond to the virus by upregulation of chemokine CXCL10.

Discussion

Chronic RV infection in HG patients is common and causes an additional disease burden. Here, we showed reduced total and memory B cell subsets and increased expression of antiviral genes in HG patients. Furthermore, the expression of antiviral genes correlated with transcription factor STAT1, which was also increased in HG patients. Also, we measured virus presence in sorted cell subsets and found that monocytes are containing rhinovirus RNA. B cells, T cells and monocytes expressed increased levels of virus induced genes.

In line with previous findings [10], we reported changes in several B lymphocyte subsets in HG patients, especially among switched B cells. While numbers of total B cell, IgA+ and IgG+ switched cells were decreased, less mature B cell subsets were normal, and IgD+ and IgM+ switched cells showed even increased numbers in HG patients. Changes in B cell subsets in infected HG patients are most likely not a result of the infection but a consequence of the CVID of these patients. As virus neutralizing IgA and IgG are crucial during RV infections, lower levels of memory cells are likely to result in stronger and prolonged infection as previously reported for HG patients [11,12].

We found increased expression of *STAT1* in PBMCs after RV infection. This is consistent with an earlier study demonstrating upregulated expression of *STAT1* in whole blood from lymphopenic CVID patients [14]. STAT1 as a central transcription factor acts downstream of the type-I-IFN receptor and induces a number interferon-stimulated genes (ISG) [16,27]. Accordingly, also several ISGs were upregulated in PBMC, including *IFITM1*, *MX1*, *IFIT1* and *IFI44L*. Interestingly, *STAT1* expression in CD19+ B cells, CD3+ T cells, and CD14+ monocytes was higher in HG patients compared with infected control group. In addition, *IFITM1* and *MX1* showed a similar tendency of higher expression in B and T cells from HG patients. Importantly, gene expression of many ISGs correlated to expression of their transcription factor *STAT1*, which may suggest that ISGs were upregulated in response to IFN-signalling via STAT1. Such an overall stronger inflammatory signaling, including type-I-interferons has been demonstrated before in HG patients and seems not to be a consequence of the Ig replacement therapy they are recieving [14]. In fact, this inflammatory response might be a directed mechanism of the immune system trying to compensate for the missing humoral response [18,28]. On the other hand, higher IFN-response might also be the simple consequence of longer virus exposure in these patients [11,12]. It is currently unknown whether higher gene expression of ISG is beneficial for the cell types we described. Interestingly, it was found that HG patients had actually low levels of expressed antiviral protein MX1 and only administering IFN-α upon RV infection led to upregulation of MX1 protein in blood and to clearance of RV [29]. Therefore, there might be a discrepancy between elevated expression of IFN-responsive genes and their translation into proteins in these patients.

PBMC and CD14+ monocytes from infected HG patients showed strong upregulation of *STAT1* and *STAT1* gene expression highly correlated to expression of the IFN-induced chemokine *CXCL10*.

Among the cell subsets studied here, CD14+ monocytes are the most efficient to collect RV particles after *in vitro* infection of PBMCs [30]. In this study, we found that monocytes carried RV-derived RNA in up to 89% of individuals that were tested positive for RV in nasal swaps while we found a positive signal only in one subject that was reported as uninfected control. We could not detect RV in PBMC samples, probably because the number of monocytes was too small to allow RV detection. The virus load seems to play a functional role in monocytes as in samples that contained more virus RNA the antiviral response genes *STAT1* and *CXCL10* were activated more strongly.

Our study has some limitations that should be considered when interpreting our results. Firstly, the numbers of subjects in each of the three groups (infected HG patients, infected controls, uninfected controls) were rather low. In addition, there were more women participating in the study and mean age of control subjects was singificantly smaller than of infected HG patients. However, we did not find significant difference in gene expression in women compared to men and gene expression was not significantly correlating with age within non-infected or infected group. Another possible limitation is, that since we study natural infections in patients who attend to the outpatient clinic, we do not know the exact date when the subjects were initially exposed to the virus. As type-I IFN response can start within hours after infection, the peak of infection and immune response might have been earlier, before the sampling. This might explain why expression of many antiviral functional genes was not as strongly induced as reported in earlier studies ([13] and unpublished data). Furthermore, we assessed *STAT1* gene expression. While increased *STAT1* expression was previously found to be a marker of upregulated antiviral responses [16], measuring the amount of phosphorylated STAT1 protein would have been more reliable to estimate the biological role of the expressed STAT1.

In conclusion, the data presented here improve our understanding of the immune response to RV mediated by B cells, T cells and monocytes in HG patients. Specifically, we demonstrated that the gene expression is most likely dysregulated in several cell types of the blood, including CD19+ B cells, CD3+ T cells and CD14+ monocytes that can play a role in chronicity of anti-viral response. Furthermore, we showed that monocytes carried RV which was linked to higher expression of *STAT1* and *CXCL10*. RV therefore seems to induce an antiviral response in circulating immune system cells from HG patients as well as healthy individuals. Our results of increased antiviral gene expression in HG patients support previous findings of prolonged inflammation and increased IFN-response in total PBMC from HG patients [14]. Further studies should cover the mechanism responsible for increased expression of ISGs and address why this is not leading to more efficient antiviral response in HG patients [29]. Since the naturally-induced IFN-response seems not effective enought for induction of functional antiviral proteins at least in some HG patients [29] it might make sense to consider IFN-treatment to further boost the antiviral response.

Author contributions

OFW, TJ and MA designed the study and wrote the manuscript; AL, LK and TJ recruited the patients and collected patient material; OFW, ÖÜ and KJ performed and analysed experiments; all authors supported and made contributions to data interpretation, and revised and approved the final version of the manuscript.

References

1. Gwaltney JM, Jr., Hendley JO, Simon G, Jordan WS, Jr. Rhinovirus infections in an industrial population. I. The occurrence of illness. *N Engl J Med.* 1966;275(23):1261-1268.
2. Miller EK, Williams JV, Gebretsadik T, et al. Host and viral factors associated with severity of human rhinovirus-associated infant respiratory tract illness. *J Allergy Clin Immunol.* 2011;127(4):883-891.
3. Hyvarinen MK, Kotaniemi-Syrjanen A, Reijonen TM, Korhonen K, Korppi MO. Teenage asthma after severe early childhood wheezing: an 11-year prospective follow-up. *Pediatric pulmonology.* 2005;40(4):316-323.
4. Jartti T, Smits HH, Bonnelykke K, et al. Bronchiolitis needs a revisit: Distinguishing between virus entities and their treatments. *Allergy.* 2019;74(1):40-52.
5. Lund RJ, Osmala M, Malonzo M, et al. Atopic asthma after rhinovirus-induced wheezing is associated with DNA methylation change in the SMAD3 gene promoter. *Allergy.* 2018;73(8):1735-1740.
6. Hasegawa K, Hoptay CE, Harmon B, et al. Association of type 2 cytokines in severe rhinovirus bronchiolitis during infancy with risk of developing asthma: A multicenter prospective study. *Allergy.* 2019;74(7):1374-1377.
7. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ (Clinical research ed).* 1995;310(6989):1225-1229.
8. Barclay WS, al-Nakib W, Higgins PG, Tyrrell DA. The time course of the humoral immune response to rhinovirus infection. *Epidemiol Infect.* 1989;103(3):659-669.
9. Alper CM, Doyle WJ, Skoner DP, Buchman CA, Cohen S, Gwaltney JM. Prechallenge antibodies moderate disease expression in adults experimentally exposed to rhinovirus strain hanks. *Clin Infect Dis.* 1998;27(1):119-128.
10. Blanco E, Perez-Andres M, Arriba-Mendez S, et al. Defects in memory B-cell and plasma cell subsets expressing different immunoglobulin-subclasses in patients with CVID and immunoglobulin subclass deficiencies. *J Allergy Clin Immunol.* 2019.
11. Kainulainen L, Vuorinen T, Rantakokko-Jalava K, Osterback R, Ruuskanen O. Recurrent and persistent respiratory tract viral infections in patients with primary hypogammaglobulinemia. *J Allergy Clin Immunol.* 2010;126(1):120-126.
12. Peltola V, Waris M, Kainulainen L, Kero J, Ruuskanen O. Virus shedding after human rhinovirus infection in children, adults and patients with hypogammaglobulinaemia. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases.* 2013;19(7):E322-327.
13. Zaas AK, Chen M, Varkey J, et al. Gene expression signatures diagnose influenza and other symptomatic respiratory viral infections in humans. *Cell host & microbe.* 2009;6(3):207-217.
14. Park J, Munagala I, Xu H, et al. Interferon signature in the blood in inflammatory common variable immune deficiency. *PLoS One.* 2013;8(9):e74893.
15. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. *Nat Rev Immunol.* 2014;14(1):36-49.
16. Lehtonen A, Matikainen S, Julkunen I. Interferons up-regulate STAT1, STAT2, and IRF family transcription factor gene expression in human peripheral blood mononuclear cells and macrophages. *J Immunol.* 1997;159(2):794-803.
17. Gonzalez-Navajas JM, Lee J, David M, Raz E. Immunomodulatory functions of type I interferons. *Nat Rev Immunol.* 2012;12(2):125-135.
18. Shulzhenko N, Dong X, Vyshenska D, et al. CVID enteropathy is characterized by exceeding low mucosal IgA levels and interferon-driven inflammation possibly related to the presence of a pathobiont. *Clinical immunology (Orlando, Fla).* 2018;197:139-153.

19. Osterback R, Tevaluoto T, Ylinen T, et al. Simultaneous detection and differentiation of human rhino- and enteroviruses in clinical specimens by real-time PCR with locked nucleic Acid probes. *J Clin Microbiol.* 2013;51(12):3960-3967.
20. Wai-Ming Lee KG, Rose Vrtis, Tressa Pappas, Fue Vang, Iris Lee, and James E. Gern. Molecular Identification and Quantification of Human Rhinoviruses in Respiratory Samples. *Rhinoviruses: Methods and Protocols, Methods in Molecular Biology.* 2015;1221:25.
21. Message SD, Laza-Stanca V, Mallia P, et al. Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci U S A.* 2008;105(36):13562-13567.
22. Levandowski RA, Ou DW, Jackson GG. Acute-phase decrease of T lymphocyte subsets in rhinovirus infection. *J Infect Dis.* 1986;153(4):743-748.
23. Maglione PJ, Gyimesi G, Cols M, et al. BAFF-driven B cell hyperplasia underlies lung disease in common variable immunodeficiency. *JCI insight.* 2019;4(5).
24. Baris S, Alroqi F, Kiykim A, et al. Severe Early-Onset Combined Immunodeficiency due to Heterozygous Gain-of-Function Mutations in STAT1. *Journal of clinical immunology.* 2016;36(7):641-648.
25. Xi Y, Finlayson A, White OJ, Carroll ML, Upham JW. Rhinovirus stimulated IFN-alpha production: how important are plasmacytoid DCs, monocytes and endosomal pH? *Clinical & translational immunology.* 2015;4(10):e46.
26. Nakagome K, Bochkov YA, Ashraf S, et al. Effects of rhinovirus species on viral replication and cytokine production. *J Allergy Clin Immunol.* 2014;134(2):332-341.
27. Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. *Nat Rev Immunol.* 2008;8(7):559-568.
28. Strannegard O, Bjorkander J, Hellstrand K, Pacsa A, Hermodsson S, Hanson LA. Interferon and beta 2-microglobulin in patients with common variable immunodeficiency or selective IgA deficiency. *International archives of allergy and applied immunology.* 1987;84(3):217-222.
29. Ruuskanen O, Waris M, Kainulainen L. Treatment of persistent rhinovirus infection with pegylated interferon alpha2a and ribavirin in patients with hypogammaglobulinemia. *Clin Infect Dis.* 2014;58(12):1784-1786.
30. Aab A, Wirz O, van de Veen W, et al. Human rhinoviruses enter and induce proliferation of B lymphocytes. *Allergy.* 2017;72(2):232-243.

Figure legends

Fig 1. Hypogammaglobulinemia patients show reduced total and memory B cells. Frozen PBMC from healthy subjects that either uninfected or infected with rhinovirus, and from rhinovirus-infected hypogammaglubulinemia (HG) patients were defrosted and stained using antibodies for cell surface markers as indicated. (A) Cell numbers of B cells from 105 total lymphocytes, (B, C, D) Cell numbers of IgM+ IgD+ non-switched B cells (B), IgM+ IgD+ CD10+ immature B cells (C), CD24high CD38high transitional B cells (D), IgD+ memory B cells (E), IgM+ memory B cells (F), and switched IgM- IgD- B cells (G) from 104 CD19+ B cells. (H) Cell numbers of switched IgA+ (left) and IgG+ B cells (right) from 103 CD19+ B cells. (I, J) Comparison of heavy chain isotype expression among the three subjects groups (I) and within an individual group (J). (K) Cell numbers shown for CD14+ monocytes and CD3+ T cells. Individual data points show cases from a total of 14 uninfected healthy subjects, 7 infected healthy subjects and 11 infected HG patients. Significant differences were calculated between uninfected and infected healthy groups, as well as between infected healthy and HG groups using two-tailed unpaired t-test, * $p \leq 0.05$, ** $p \leq 0.01$; *** $p \leq 0.001$, error bars depicts SD. HG = hypogammaglobulinemia patients.

Fig 2. Expression of virus-response related genes in PBMC and different blood cell subsets. Frozen Frozen PBMC from healthy subjects that either uninfected or infected with rhinovirus, and from rhinovirus-infected hypogammaglubulinemia (HG) patients were defrosted and CD19+, CD14+ and CD3+ cells were sorted using FACS. (A-D) Expression of transcription factor gene *STAT1* and downstream antiviral genes *IFITM1* and *MX1* in full PBMC (A), in CD19+ B cells (B), CD14+ monocytes (C) , and CD3+ T cells (D). Individual data points show cases from a total of 14 uninfected healthy subjects, 7 infected healthy subjects and 11 infected HG patients. Significant differences were calculated between uninfected and infected healthy group, as well as between infected healthy and HG group using two-tailed unpaired t-test, * $p \leq 0.05$, ** $p \leq 0.01$; *** $p \leq 0.001$, exact p-values noted when $p < 0.1$, values are means ± SEM. HG = hypogammaglobulinemia patients.

Fig 3. Expression of antiviral genes correlate with transcription factor STAT1-expression. Frozen Frozen PBMC from healthy subjects that either uninfected or infected with rhinovirus, and from rhinovirus-infected hypogammaglubulinemia (HG) patients were defrosted and CD19+, CD14+ and CD3+ cells were sorted using FACS. (A, B) Expression antiviral gene IFITM1 (A) and MX1 (B) correlated to expression of *STAT1 for CD19+, CD14+ and CD3+ cells.* Individual data points show cases from a total of 14 uninfected healthy subjects, 7 infected healthy subjects and 11 infected HG patients. Significant correlation was calculated for all data points using Pearson correlation. HG = hypogammaglobulinemia patients.

Fig 4. Monocytes carry rhinovirus RNA after natural infection with rhinovirus. CD14+ monocytes were sorted from frozen PBMC from healthy subjects that either uninfected or infected with rhinovirus, and from rhinovirus-infected HG patients. (A, B, C) PCR using pan-rhinovirus primers shows occurence of rhinovirus-derived cDNA in monocytes from healthy subjects that either uninfected (A), infected with rhinovirus (B), and from rhinovirus-infected HG patients (C). (D) Total number and percentage of infected samples shown for individual groups. Individual bands show cases from a total of 14 uninfected healthy subjects, 7 infected healthy subjects and 11 infected HG patients. HG = hypogammaglobulinemia patients; a, b, c = different timepoints of individual donors. Arrow marks expected band size..

Fig 5. Increased expression of antiviral gene STAT1 and chemokine CXCL10 correlates with higher virus load in circulating monocytes. Relative rhinovirus load from samples of uninfected control, infected control and infected HG subjects was calculated by comparing band intensities to positive control of each gel. Samples were grouped: negative/low (band intensity ≤ 45% of positive control), intermediate (45-90 %) and high (≥.90 %). Expression of transcription factor *STAT1* (A) and chemokine *CXCL10* (B) is shown for samples of different band intensity. Significant differences were calculated between groups using Kruskal-Wallis. * $p \leq 0.05$, ** $p \leq 0.01$. HG = hypogammaglobulinemia patients.

Supplementary figure legends

Fig S1. Gating strategy used for sorting CD19+ B cells, CD14+ moncytes and CD3+ T cells. Frozen PBMC from healthy subjects that were either uninfected or infected with rhinovirus, and from rhinovirus-infected hypogammaglubulinemia (HG) patients were defrosted; CD19+, CD14+ and CD3+ cells were sorted using FACS.

Fig S2. Gating strategy used for B cell subsets. Frozen PBMC from control subjects that were either uninfected or infected with rhinovirus, and from rhinovirus-infected hypogammaglubulinemia (HG) patients were defrosted and stained using antibodies for cell surface markers as indicated. B cell subsets were gated from viable CD19+ B cells. Gating shown for infected control (upper panel) and for infected HG patient (lower panel).

Fig S3. Increased range of κ-light chain expression in hypogammaglobulinemia patients. Frozen PBMC from healthy subjects that either uninfected or infected with rhinovirus, and from rhinovirus-infected hypogammaglubulinemia (HG) patients were defrosted and stained using antibodies for cell surface markers as indicated. B cell subsets were gated from CD19+ B cells. **A - G**, Kappa-light chain expression shown for total B cells (**A**), IgD+ gM+ B cells (**B**), IgD+ IgM+ CD10+ naive B cells (**C**),

switched single Ig-expressing IgM+ (**D**), IgD+ (**E**), IgA+ (**F**), and IgG+ (**G**) B cells. Individual data points show cases from a total of 14 uninfected healthy subjects, 7 infected healthy subjects and 11 infected HG patients. Significant differences were calculated between uninfected and infected healthy groups, as well as between infected healthy and HG groups using two-tailed unpaired t-test, * $p \leq 0.05$, ** $p \leq 0.01$; *** $p \leq 0.001$, error bars depicts SD. HG = hypogammaglobulinemia patients.

Fig S4. Expression of virus-response related genes *ITIF1*, *IFI44L* and *DDX58* in PBMC and different blood cell subsets. Frozen PBMC from healthy subjects that either uninfected or infected with rhinovirus, and from rhinovirus-infected hypogammaglubulinemia (HG) patients were defrosted and CD19+, CD14+ and CD3+ cells were sorted using FACS. **A - D**, Expression of transcription factor gene *STAT1* and downstream antiviral genes *IFITM1*, *MX1* and *DDX58* in full PBMC (**A**), in CD19+ B cells (**B**), CD14+ monocytes (**C**) , and CD3+ T cells (**D**). Individual data points show cases from a total of 14 uninfected healthy subjects, 7 infected healthy subjects and 11 infected HG patients. Significant differences were calculated between uninfected and infected healthy group, as well as between infected healthy and HG group using two-tailed unpaired t-test, * $p \leq 0.05$, ** $p \leq 0.01$; *** $p \leq 0.001$, exact p-values noted when $p < 0.1$, values are means ± SEM. HG = hypogammaglobulinemia patients.

Fig S5. Grading of gel band intensities for individual samples. Gel band intensity for individual samples from Fig 5 of different subject groups assessed by *Evolution-Capt Edge* software (Vilber Lourmat).