Immunobiology 226 (2021) 152151

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

RvD1 treatment during primary infection modulates memory response increasing viral load during respiratory viral reinfection

Deise do Nascimento de Freitas [a], Caroline Marinho Franceschina [a], Douglas Muller [b], Gabriel T. Hilario [b], Rodrigo B. Gassen [c], Tiago Fazolo [b], Valéria de Lima Kaminski [d], José Artur Bogo Chies [h], Fábio Maito [e], Krist Helen Antunes [a,g], Rafael F. Zanin [f], Luiz Carlos Rodrigues Jr [b], Ana Paula Duarte de Souza [a,g,*]

[a] Laboratory of Clinical and Experimental Immunology, School of Health and Life Science, Pontifical Catholic University of Rio Grande do Sul (PUCRS), Porto Alegre, RS, Brazil
[b] Federal University of Health Sciences of Porto Alegre (UFSCPA), Porto Alegre, RS, Brazil
[c] Schuster Family Transplantation Research Center, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
[d] Applied Immunology Laboratory, Postgraduate Program in Biotechnology, Institute of Science and Technology – ICT, Federal University of São Paulo - UNIFESP, Brazil
[e] Laboratory of Histology, School of Health and Life Science, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre 90610-000, RS, Brazil
[f] Department of Health and Human Development, La Salle University, Canoas, RS, Brazil
[g] Infant Center, School of Medicine PUCRS, Porto Alegre, RS, Brazil
[h] Laboratory of Immunogenetics and Immunobiology, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
D1 Resolvin
Memory CD8+ T cells
mTOR
Respiratory syncytial virus

ABSTRACT

Resolvin D1 (RvD1), which is biosynthesized from essential long-chain fatty acids, is involved in anti-inflammatory activity and modulation of T cell response. Memory CD8+ T cells are important for controlling tumor growth and viral infections. Exacerbated inflammation has been described as impairing memory CD8+ T cell differentiation. This study aimed to verify the effects of RvD1 on memory CD8+ T cells *in vitro* and *in vivo* in a respiratory virus infection model. Peripheral blood mononuclear cells were treated at different time points with RvD1 and stimulated with anti-CD3/anti-CD28 antibodies. Pre-treatment with RvD1 increases the expansion of memory CD8+ T cells. The IL-12 level, a cytokine described to control memory CD8+ T cells, was reduced with RvD1 pre-treatment. When the mTOR axis was inhibited, the IL-12 levels were restored. In a respiratory virus infection model, Balb/c mice were treated with RvD1 before infection or after 7 days after infection. RvD1 treatment after infection increased the frequency of memory CD8+ T cells in the lung expressing *Il4*, *Il10*, and *Ifng*. During reinfection, RvD1-treated and RSV-infected mice present a high viral load in the lung and lower antibody response in the serum. Our results show that RvD1 modulates the expansion and phenotype of memory CD8+ T cells but contributed to a non-protective response after RSV reinfection.

1. Introduction

Specialized pro-resolution mediators (SPM) have anti-inflammatory activity and are one of the key mediators of the resolution phase of inflammation. SPM is enzymatically derived from arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), which are long-chain fatty acids (Simopoulos, 2002; Bannenberg and Serhan, 2010; Schmitz and Ecker, 2008). The main SPM families are resolvins, protectins, maresins, and lipoxins (Isobe et al., 2013). E-series resolvins (RvE1, RvE2, and RvE3) are derived from EPA, and D-series resolvins (RvD1-6) are derived from DHA. Resolvin D1 (RvD1) is one of the main mediators in the anti-inflammatory response (Levy et al., 2001; Serhan, 2004; Serhan et al., 2004; Hong and Lu, 2013), a role extensively evidenced in the literature. RvD1 reduces infiltration of neutrophils and decreases the levels of inflammatory cytokines, which are important in the pro-resolution phase (Buckley et al., 2014). Also, RvD1 decreases the production of IL-12 by monocytes (Gu et al., 2016). RvD1 treatment decreases symptoms in animal models of autoimmune

* Corresponding author at: Laboratory of Clinical and Experimental Immunology, School of Health and Life Science, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre 90610-000, RS, Brazil.
E-mail address: ana.duarte@pucrs.br (A.P. Duarte de Souza).

https://doi.org/10.1016/j.imbio.2021.152151
Received 30 March 2021; Received in revised form 22 October 2021; Accepted 23 October 2021
Available online 31 October 2021

diseases (Zhang et al., 2008). In a model of multiple sclerosis, RvD1 treatment reduces T cell response and cytokine production, inducing M2 differentiation (Poisson et al., 2015). Treatment with an analog of RvD1 also suppresses host T-cell allosensitization in mice (Hua et al., 2014). Also, RvD1 induces the differentiation of regulatory T cells and the production of IL-10 (Chiurchiù et al., 2016). However, the role of RvD1 in memory CD8+ T cell response is unknown.

Memory CD8+ T cell response is essential to lifelong immunity protection, and it has been the focus of various immunotherapy strategies and vaccines for intracellular pathogens and tumors (Joshi et al., 2007; Osborne and Abraham, 2010; Pham et al., 2009; Valbon et al., 2016; Jarjour et al., 2021). The key characteristics that define memory T cells include long-lived homeostatic proliferation driven by cytokines, rapid proliferative capacity, and cytotoxic activity after encountering the cognate epitope.

Respiratory syncytial virus (RSV), is the leading cause of respiratory infection in children, and reinfection with RSV is common (Bont et al., 2002). Nevertheless, the mechanism associated with the development of protective memory against respiratory viral reinfection is not fully elucidated, making this an appealing model to investigate memory (Ascough et al., 2018).

The inflammatory milieu affects different aspects of memory CD8+ T cells' response before, during, and after their reactivation by the antigen (Osborne and Abraham, 2010; Valbon et al., 2016). Studies have demonstrated that the development of memory CD8+ T cells is enhanced after immunization with a low-inflammatory environment (Joshi et al., 2007; Pham et al., 2011). Inflammatory cytokines induced by specific pathogens can also dictate the antigen sensitivity of memory CD8+ T cells (Richer et al., 2012). For example, it has been reported that IL-12 hinders the formation of CD8+ T cell memory that lasts a long time in the mouse model (Pearce and Shen, 2007). In contrast, IL-10 production by regulatory CD4 T cells during the resolution phase of infection is important for the differentiation of memory CD8+ T cells (Laidlaw et al., 2015). Understanding all the factors in the inflammatory environment that dictate the generation and maintenance of memory CD8+ T cell response is of great translational interest for the development of novel vaccines and immunotherapeutic advances (Joshi et al., 2007; Pham et al., 2011).

Given the importance of the anti-inflammatory response and memory CD8+ T cell generation, we hypothesized that RvD1 might have an important effect on the memory CD8+ T cell response. In the current study, we investigate the effects of RvD1 on human memory CD8+ cells *in vitro* and in a murine model of respiratory virus infection.

2. Materials and methods

2.1. Subjects of study

Peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteers (n = 20) between 20 and 40 years old of both sexes. Ten milliliters of blood were collected from donors after signing an informed consent form. All procedures following the Declaration of Helsinki and were approved by the PUCRS Research Ethics Committee (844.206/2014).

2.2. In vitro RvD1 treatments and cellular stimulation in PBMC

PBMCs were isolated using Ficoll (Sigma-Aldrich®). Cells (10^6 /well) were seeded in 96 well-plate in RPMI 5% of FBS (Gibco) and stimulated with 2 μg/ml anti-CD3/anti-CD28 antibodies (TCR stimulation) (BD Bioscience®) and/or treated with 10 nM of RvD1 at multiple time points (30 min before the stimulus or 6 or 12 or 24 h after the stimulus). To perform the treatment with RvD1 we diluted the stock (100 μg/μl - Cayman Chemical®) in RPMI media (1/1000) and used 7.5 μl per well. The concentration of 10 nM was established according to (Chiurchiù et al., 2016). The media was replenished after RvD1 treatment before or after the stimulus and on the third day of culture. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 for 7 days, as previously described by Geginat et al. (Geginat et al., 2003). Alternatively, 1 h before RvD1 treatment, cells were incubated with 10 ng/mL rapamycin (Cell Signaling®). Cells were also treated with anti-IL2 receptor antibody (R&D Systems®) before TCR stimulation. Cell supernatant was collected after 3 days of culture for cytokine assay. CD8+ T cell phenotypes and viability was assessed after 7 days with Fixable Viability Dye eFluorTM 780 (Invitrogen®) according to the manufacturer's instructions. To characterize the phenotype of the CD8+ human cells, we used the following surface antibodies: anti-CD8 (clone RPA-T8), anti-CD45RA (clone HI100), anti-CD45RO (clone UCHL-1), anti-CD27 (clone M-T271), and granzyme B (clone GB11) (BD Biosciences®). Samples were acquired by FACS CANTO II flow cytometry (BD Bioscience®). The FlowJo software (TreeStar®) was used for data analysis. IL-12p40 cytokine levels in the cell supernatant were analyzed using an ELISA kit (ImmunoTools®) according to the manufacturer's instructions.

2.3. Mice

Balb/c mice were obtained from the Animal Facility of Universidade Federal Ciências da Saúde de Porto Alegre (UFCSPA). Female animals (6–8 weeks old) were used for experiments and housed at the animal facility with water and food *ad libitium*. All procedures involving animals were realized according to protocols approved by the Committee on Animal Use of UFSCPA (518/17).

2.4. Virus

RSV A strain (line A2) was kindly donated by Fernando Polack (Fundacion Infant, Argentina). RSV was cultivated in Vero cells (ATCC CCL81) using Optimem medium containing 2% fetal bovine serum (FBS). Viral plaque-forming units (PFU) were identified with a mouse anti-RSV monoclonal antibody (EMD-Millipore®).

2.5. RSV infection and RvD1 treatment in mice

Mice were anesthetized on day 0 with isoflurane, and afterward infected with 10^7 PFU RSV virus intranasally. We diluted the stock solution of RvD1 in PBS (1/200) and administered 100 ng of RvD1 per mice intraperitoneally in 200 μl (Cayman Chemical®). The dose and the administration route were chosen according to (Chiurchiù et al., 2016). RvD1 treatment was performed 30 min before the intranasal RSV infection (10^7 PFU RSV) or after 7 days of infection. The controls were infected and received PBS (Phosphate Buffered saline) treatment at the same time as the experimental groups. Alternatively ethanol (solvent RvD1) was diluted in PBS the same way as RvD1, used as a control. The CD8+ memory response was analyzed 14 after infection. Infected mice after 14 days were challenged with 10^7 PFU of RSV virus intranasally. The response was analyzed after 8 h. The serum was collected, and the spleen and lymph nodes were harvested for cell staining. Cell from lung tissue was obtained using the collagenase D solution for 1 h at 37 °C in 5% CO_2. Cells were analyzed by flow cytometry and reverse transcription-polymerase chain reaction (RT-PCR).

2.6. Murine memory cells staining

The murine cells were incubated with a specific tetramer to the peptide of the M2 protein (SYIGSINNI) from RSV (M282–90) and were stained with the following surface antibodies: anti-CD8+ (Percp and PE-Cy7) (clone 53,67), anti-CD127 (PE-Cy7) (clone SB/199), anti-KLRG1 (APC) (clone 2FI), anti-CD103 (PE) (clone: M290), anti-CD44 (PerCp) (clone: IM7), anti-CD62L (APC) (clone: MEL-14) (BD Biosciences®), anti-IL-10 (APC) (clone JES5-16E), anti-IL4 (clone 11B11), and anti-IFN-γ (ALEXA 647) (clone XMG1.2) (BD Biosciences®). Samples were acquired by FACS CANTO II flow cytometry (BD Bioscience®). The FlowJo

software (TreeStar®) was used for data analysis.

2.7. Murine lung memory CD8+ T cells sorting

After 14 days of infection, lung cells were incubated with mouse FcBlock at 4 °C for 20 min and stained with anti-CD8+ (Percp) (clone 53.67) and anti-CD127 (PE) (clone: SB/199) for 30 min. The cells were sorted to gather the population of memory CD8+ T cells (CD8 + $CD127^{high}$) using a FACS Aria II (BD Bioscience®), achieving 90% purity. Total RNA was extracted, and cDNA was synthesized for gene expression analysis.

2.8. Gene expression of memory cells

cDNA of purified memory cells was used to determine the expression levels of IL-4, IL-10, IL-13, IL-5, IFN-ɣ, GATA-3, T-bet, and Blimp-1. The following primers were used: *Il4* (forward primer: 5′-GGCATTTTGAACGAGGTCACA-3′; reverse primer: 5′-GACGTTTGGCACATCCATCTC-3′); *Il10* (forward primer: 5′-AAGGGTTACTTGGGTTGCCA-3′; reverse primer: 5′-CCTGGGGCATCACTTCTACC-3′); *Il5* (forward primer: 5′-AGCACAGTCGTGAAAGAGACCTT-3′ reverse primer: 5′-TCCAATGCATAGCTGGTGATTT-3′); *Il13* (forward primer: 5′GGAGCTGAGCAACATCACACA-3′ reverse primer: 5′-GGTCCTGTAGATGGCATTGCA-3′); *Gata3* (forward: 5′-CAGCTCTGGACTCTTCCCAC-3′ reverse: 5′-GTTCACACACTCCCTGCCTT-3′); *Infg* (forward primer: 5′-TCAAGTGGCATAGATGTGGAAGAA-3′; reverse primer: 5′-TGGCTCTGCAGGCTTTTCATG-3′); *Tbx21* (forward primer: 5′-GCCAGGGAACCGCTTATATG-3′ reverse primer: 5′-GACGATCATCTGGGTCACATTGT-3′); *Zpf683* (Forward primer: 5′-GTCGCGGAGACGCAAGAGCA-3′; Reverse primer: 5′-CTTGGGGGCAGCCAAGGTCG-3′); e *B2M* (forward primer: 5′-CCCCAGTGAGACTGATACATACG-3′ reverse primer: 5′-CGATCCCAGTAGACGGTCTTG-3′) as endogenous control genes. The PCR conditions were according to the SYBR Green/ROX qPCR Master Mix protocols (Thermo Fisher Scientific®). The viral load of the samples was calculated using the StepOnePlus system (Applied Biosystems®). Gene expression analyses were done using the 2ˆ-ΔCt method.

2.9. Viral load analysis

Total RNA was extracted from the lungs of mice with TRIzol® Reagent (Invitrogen®). The cDNA was synthesized by Kit GoScript Reverse Transcriptase (PROMEGA®). The quality of cDNA in each sample was tested for the amplification of endogenous gene B2M (beta-2-microglobulin) with specific primers of TaqMan Assay (Applied Biosystems®). The samples that were not amplified to B2M were excluded. The gene encoding the RSV protein F was amplified using specific primers and probes (forward 5′-AACAGATGTAAGCAGCTCCGTTATC-3′, reverse 5′-CGATTTTTATTGGATGCTGTACATTT-3′and probe 5′-FAM/TGCCATAGCATG ACACAATGGCTCCT-TAMRA/-3′) by RT-PCR. For the standard curve, we performed a 10-fold serial dilution of a plasmid with RSV F protein sequence. Viral load values were calculated relative to the weight of the lung portion (copies per gram of lung).

2.10. Production of the F protein

Purified plasmid DNA (pGen2.1: RSV F Protein) was used for the transfection of HEK293 cells (Human embryonic kidney) using Lipofectamine 3000 reagent (ThermoFisher Scientific®). The transfected cells were selected using a geneticin antibiotic (G418). RSV F protein was purified using an anti-Flag column and confirmed by Western blot.

2.11. Quantification of RSV-specific IgG

Anti-RSV-specific IgG measurements were done using the ELISA method in mice serum. A 96-well plate was sensitized with the RSV F protein, produced as described above, overnight. Mice serum was added in 1/10, 1/100, and 1/500 dilutions, and incubated for 3 h at 37 °C. The plate was washed and incubated with HRP-conjugated rabbit anti-mouse IgG (Invitrogen®) for 1 h at room temperature. The plate was washed, and TBM (Life Technologies®) was added for 30 min. The absorbance was measured at a wavelength of 450 nm using an EZ Read 400 Microplate reader (Biochrom®).

2.12. Statistical analysis

Data were tested for normal distribution using the Kolmogorov-Smirnov test. Different groups were analyzed using one-way ANOVA (multiple groups) with a post hoc Bonferroni's test for parametric results and a Friedman test followed by Dunn's multiple comparisons test for non-parametric results. To compare two groups, we used the *t*-test for parametric data and the Mann-Whitney test for non-parametric data. GraphPad Prism 6.0 (San Diego, CA, USA) was used for data analysis. Results were considered significant when the p-value less than 0.05 ($p < 0.05$).

3. Results

3.1. RvD1 increases the percentage of memory CD8+ T cells.

To explore the role of the pro-resolution mediators on human memory CD8+ T cells, we treated PBMC with RvD1 at different time-points, as described in Fig. 1A (30 min before TCR stimulation, or 6 h, or 12 h, or 24 h after TCR stimulation). After 7 days of culture, cells were analyzed to characterize the phenotype of memory CD8+ T cells ($CD45RO^{+}CD45RA^{-}CD27^{+}$), naïve cells ($CD45RO^{-}CD45RA^{+}CD27^{+}$), and effector cells ($CD45RO^{-}CD45RA^{+}CD27^{-}$) (Fig. 1B) (H, P.-G., A.-C. D, F.-V. H, M. JJ, L.-G. JS., 2005). RvD1 treatment 30 min before TCR stimulation induced a significant increase in the percentage of memory CD8+ T cells (Fig. 1C and F). There was no significant difference between RvD1 treated and untreated cells regarding naïve and effector CD8+ T cell phenotypes (Fig. 1 C–E). RvD1 did not interfere in the viability of CD8+ T cells (Fig. 1 G), nor the secretion of granzyme B (Fig. 1 H and I).

3.2. RvD1 induces human memory CD8+ T cell expansion depending on the mTOR pathway and decreases IL-12p40 production

Phosphoinositide 3-kinase, serine/threonine-protein kinase, the mammalian target of rapamycin (PI3K/AKT/mTOR) pathway, is important to cell proliferation, survival, and memory cells differentiation (Araki et al., 2010; Pompura and Dominguez-Villar, 2018). To investigate the role of the mTOR pathway in the RvD1-mediated expansion of memory cells, we added an mTOR inhibitor (rapamycin) in the PBMC culture before RvD1 treatment and TCR stimulation. Our results indicated that the effect of RvD1 in memory CD8+ T cell expansion is dependent on the mTOR pathway (Fig. 2 A-B). Monocytes, dendritic cells, macrophages, and B cells produce the cytokine IL-12, which modulates memory CD8+ T response (Raeber et al., 2018; Martins Queiroz-Junior et al., 2010; Vignali and Kuchroo, 2012; Gee et al., 2009). RvD1 treatment decreased IL-12p40 cytokine production of PBMC after 3 days of culture (Fig. 2C). Using rapamycin before RvD1 treatment and TCR stimulation, IL-12p40 production was restored (Fig. 2C). To confirm that the absence of IL12-p40 plays a role in the increased frequency of memory CD8+ T cells, the anti-IL12p40 receptor antibody was used. The effect of IL-12 inhibition in the frequency of memory cells was similar to the increased effect mediated by RvD1 (Fig. 2D and E). Using the anti-IL12p40 receptor, IL12-p40 levels were significantly lower (Fig. 2F). Accordingly, IL12-p40 levels were inversely associated with the frequency of memory CD8+ T cells (Fig. 2G). Our results suggest that RvD1 treatment acts via the mTOR pathway reduces the levels of IL12-p40 and increases the frequency of

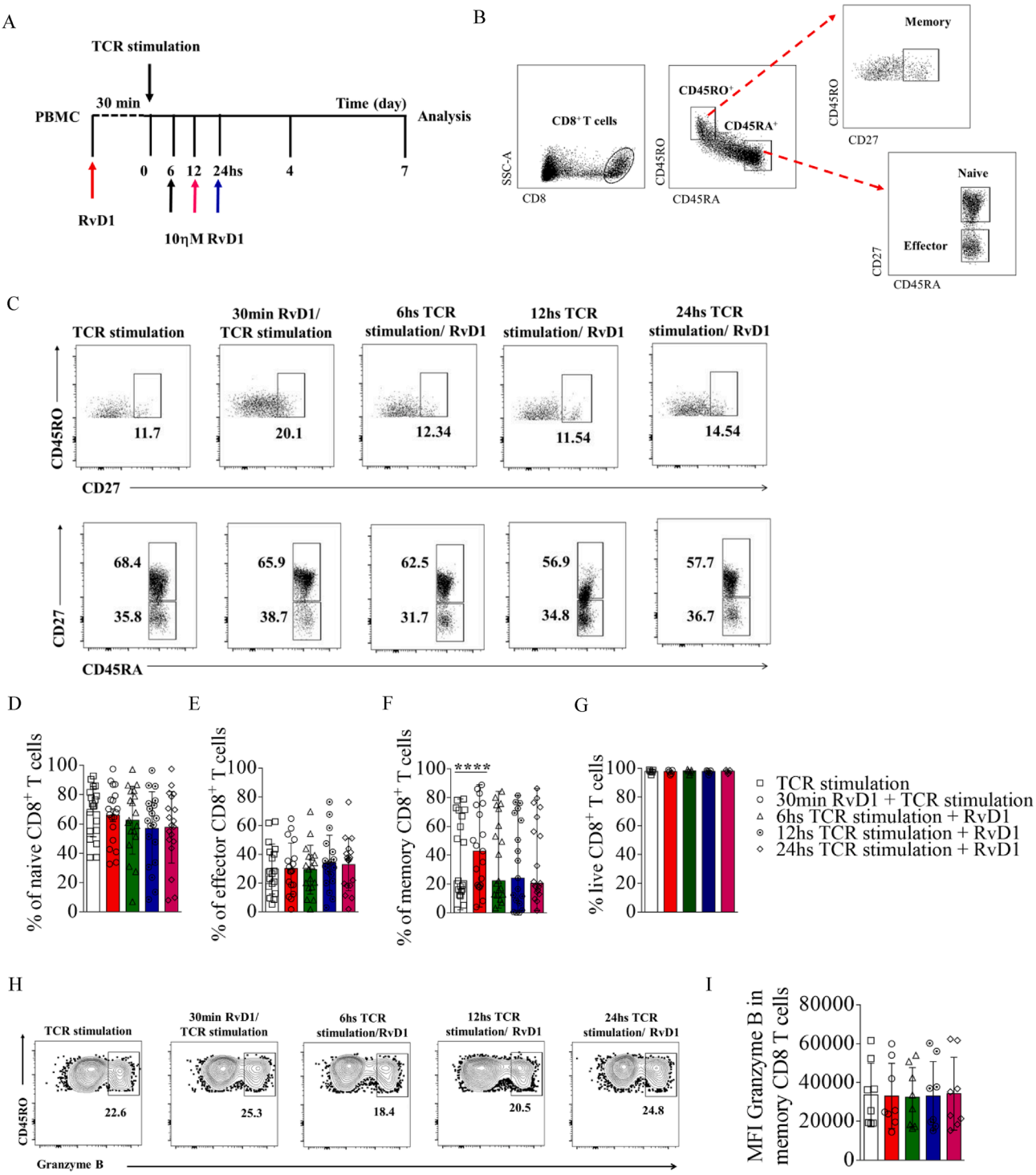

Fig. 1. RvD1 increases the expansion of human memory CD8+ T cells. Peripheral blood mononuclear cells (PBMC) were treated 10ηM RvD1 for 30 min before of stimulus of 2 μg/ml anti-CD3/anti-CD28 (TCR stimulation). Other groups were treated 6, or 12 or 24 h after stimulation. CD8+ T cells were evaluated after 7 days of culture. **(A)** Experimental design of RvD1 treatment *in vitro*. **(B)** Representative dot-plots are shown gate strategy to analysis of human naïve, effector and memory CD8+ T cells population. **(C)** Dot plots are demonstrated the frequency of naïve, effector and memory CD8+ T cells. **(D)** Frequency of naive ($CD45RA^+CD45RO^-CD27^+$) CD8+ T cells. **(E)** Frequency of effector ($CD45RA^+CD45RO^-CD27^-$) CD8+ T cells. **(F)** Frequency of memory ($CD45RA^-CD45RO^+CD27^+$) CD8+ T cells. These data are representative of 20 healthy volunteers. **(G)** Percentage of live CD8+ T cells after 7 days of culture. **(H)** Dot-plot has showed of frequency of granzyme B from memory CD8+ T cells. **(I)** Frequency of CD8 + $CD45RA^-CD45RO^+CD27^+$ granzyme B^+ T cells. These data are presented as mean ± SD (n = 8). These data were from four and three independent experiments. Statistical analysis was realized by using One Way ANOVA test with Friedman test followed by Dunn's multiple comparisons test, ****$p < 0.0001$.

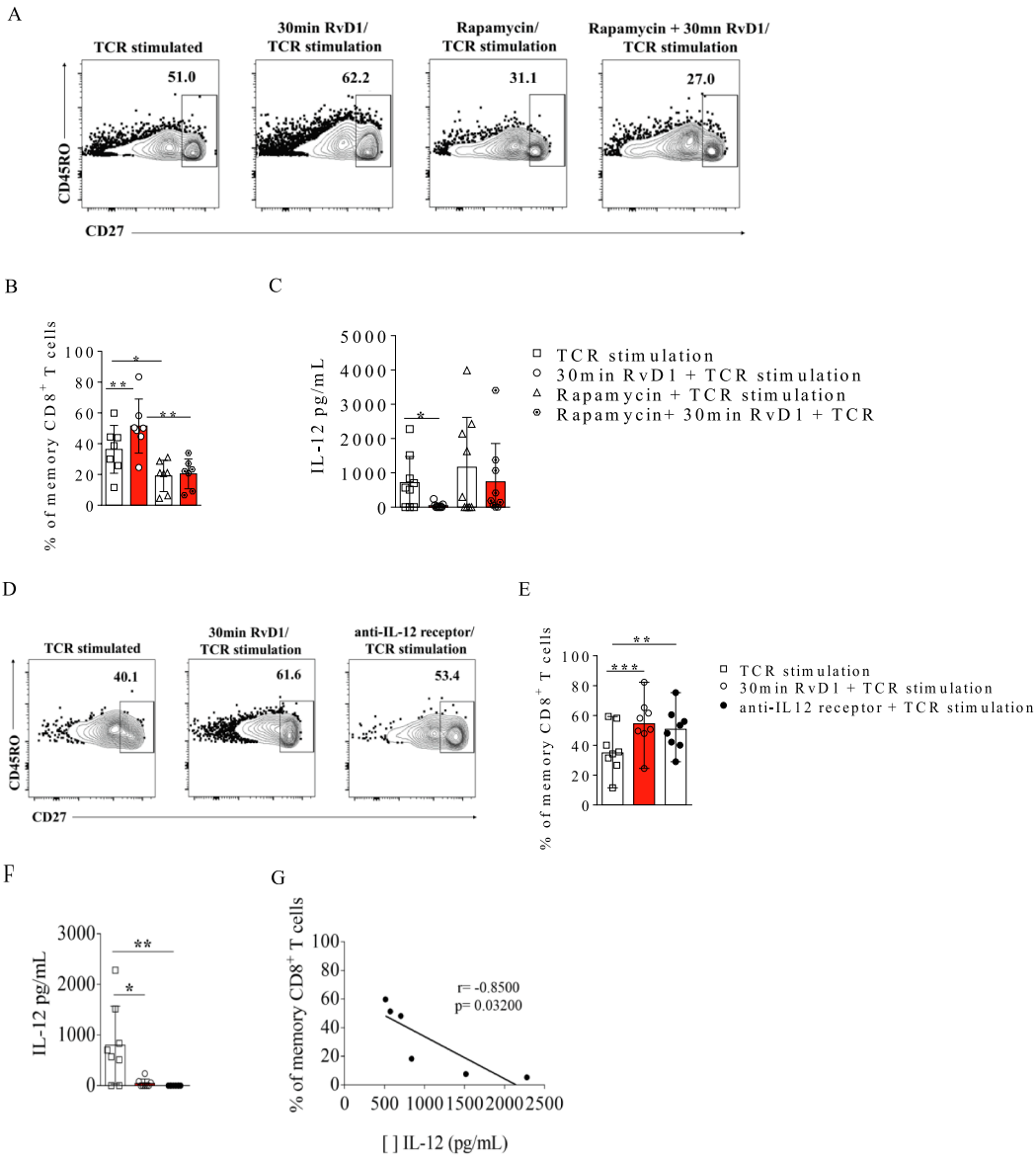

Fig. 2. RvD1 expands human memory CD8+ T cells depending on mTOR pathway activation and decreases IL-12 cytokine. PBMC incubation with 20 ηg/ml of rapamycin 1-hour prior RvD1 treatment for 30 min and TCR stimulation. After seven days of culture, cells were evaluated by flow cytometry. **(A)** Dot-plot showing the frequency of memory CD8+ T cells. **(B)** Frequency of memory CD8+ T cells after PBMC treatment with rapamycin. **(C)** Analyze IL-12p40 cytokine levels in cell culture supernatants after 72 h by ELISA assay. These data are introduced as mean ± SD (n = 10). **(D)** Dot-plot showing the frequency of memory CD8+ T cells analyzed by flow cytometry. **(E)**Frequency of memory CD8+ T cells after PBMC incubation with 1 μg/μl of anti-IL-12 receptor or RvD1 prior 30 min to TCR stimulation and cultured for seven days. **(F)** Analysis of IL-12p40 levels in cell culture supernatants collected in 72hs of stimulation. **(G)** Correlation of IL12p40 levels with the frequency of memory CD8+ T cells. These data are introduced as mean ± SD (n = 8). Data were performed in two independent experiments. Statistical analyzes were realized by ANOVA test followed by Bonferroni's multiple comparisons test, Friedman test followed by Dunn's multiple comparisons test and the correlation was analyzed by Pearson r test, * $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

human memory CD8+ T cells.

3.3. RvD1 treatment during primary respiratory infection modulates the response of memory CD8+ T leading to an increased viral load during secondary infection

We further investigated the role of RvD1 on memory CD8+ T cells using a murine model of respiratory virus infection. Balb/c mice were treated intraperitoneally with RvD1 30 min before intranasal RSV infection, and the CD8+ T cell response was analyzed after 14 days (Fig. 3A). Our results demonstrate that pre-treatment with RvD1 decreases both non-specific memory precursors CD8+ T cell ($CD8^{+}Dump^{-}CD127^{high}KLRG1^{low}$) as well as the RSV-specific memory precursors cell ($CD8^{+}Dump^{-}Tet^{(82\text{-}90)+}CD127^{high}KLRG1^{low}$) frequency in the lung (Fig. 3B–C and F-G). There was no difference in the non-specific and specific memory CD8+ T cell populations in the lymph node and spleen (Fig. 3B, D-E, F, and H–I).

The resolution of inflammation during the contraction phase of CD8+ T memory, after TCR engagement, favors maturation of memory cells (Laidlaw et al., 2015). Mice were then infected and after 7 days were treated with RvD1 (Fig. 4A). RvD1-treated mice presented an increased frequency of non-specific memory precursors CD8+ T cells ($CD8^{+}Dump^{-}CD127^{high}KLRG1^{low}$) and specific memory precursors CD8+ T cells ($CD8^{+}Dump^{-}Tet^{(82\text{-}90)+}CD127^{high}KLRG1^{low}$) against of virus in the lung (Fig. 4 B-C and G-F). The treatment did not influence the response of non-specific and specific memory precursors CD8+ T

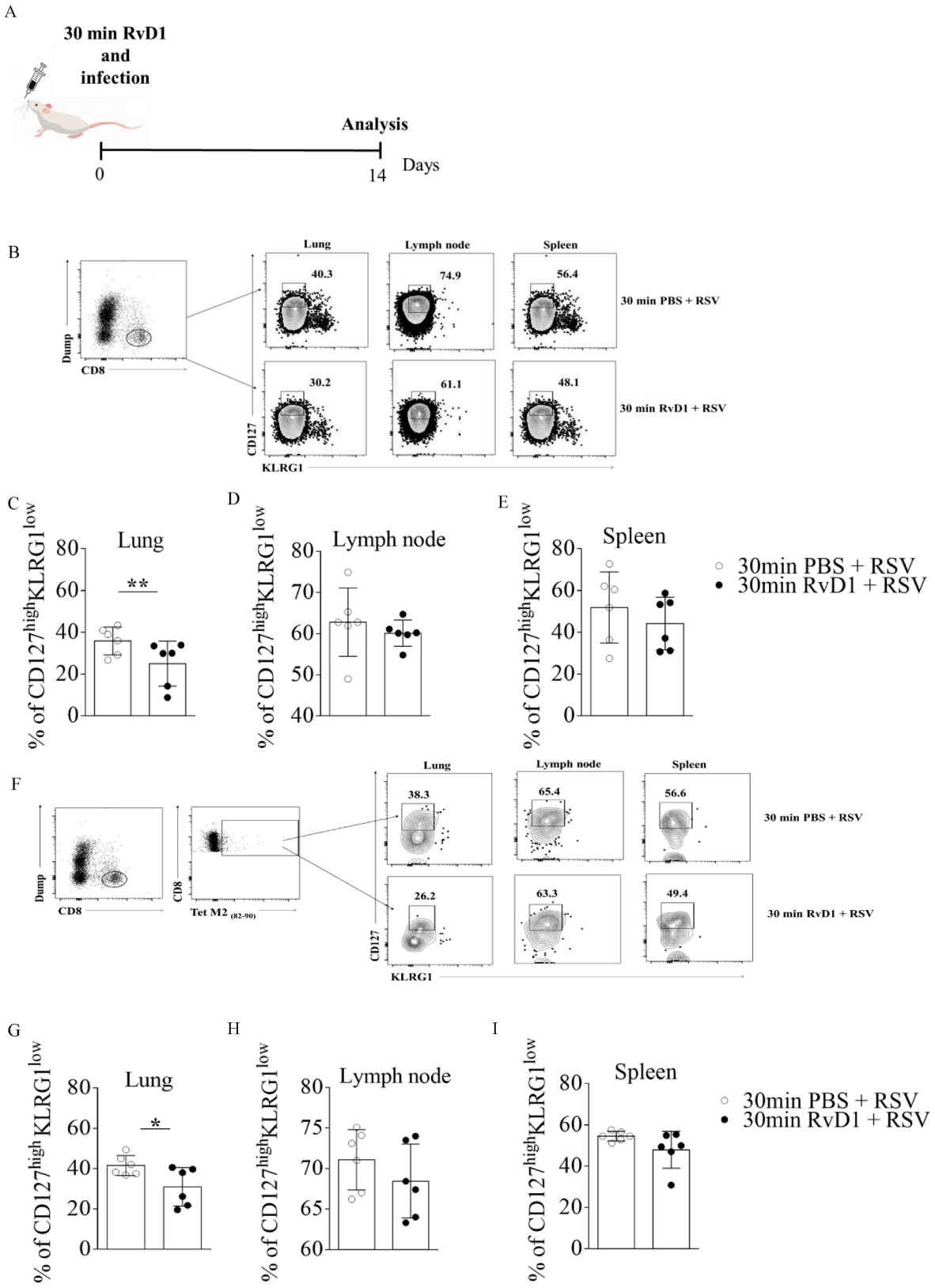

Fig. 3. RvD1 pretreatment decreases memory CD8+ T cells response in the RSV infection. **(A)** Experimental design of 30 min of RvD1 treatment before of RSV infection. **(B)** Dot-plot showed the gates of non-specific-RSV memory CD8+ T cells in pre-treated and infected mice. **(C-E)** Frequency of non-specific memory T cells (CD8 + Dump^{-}CD127^{high}KLRG1^{low}) in the lung, lymph node, and spleen from infected and RvD1 treated mice. **(F)** Dot-plot showed the gates of specific-RSV memory CD8+ T cells in pre-treated and infected mice. **(G-I).** Frequency of RSV-specific memory CD8+ T cells (CD8 + Dump^{-}Tet$_{(82-90)}^{+}$CD127^{high}KLRG1^{low}) in the lung, lymph node, and spleen from pre-treated and infected mice. These data were performed in two independent experiments (n = 6 mice/groups). The analysis statistical has determined by the T test, * $p < 0.05$.

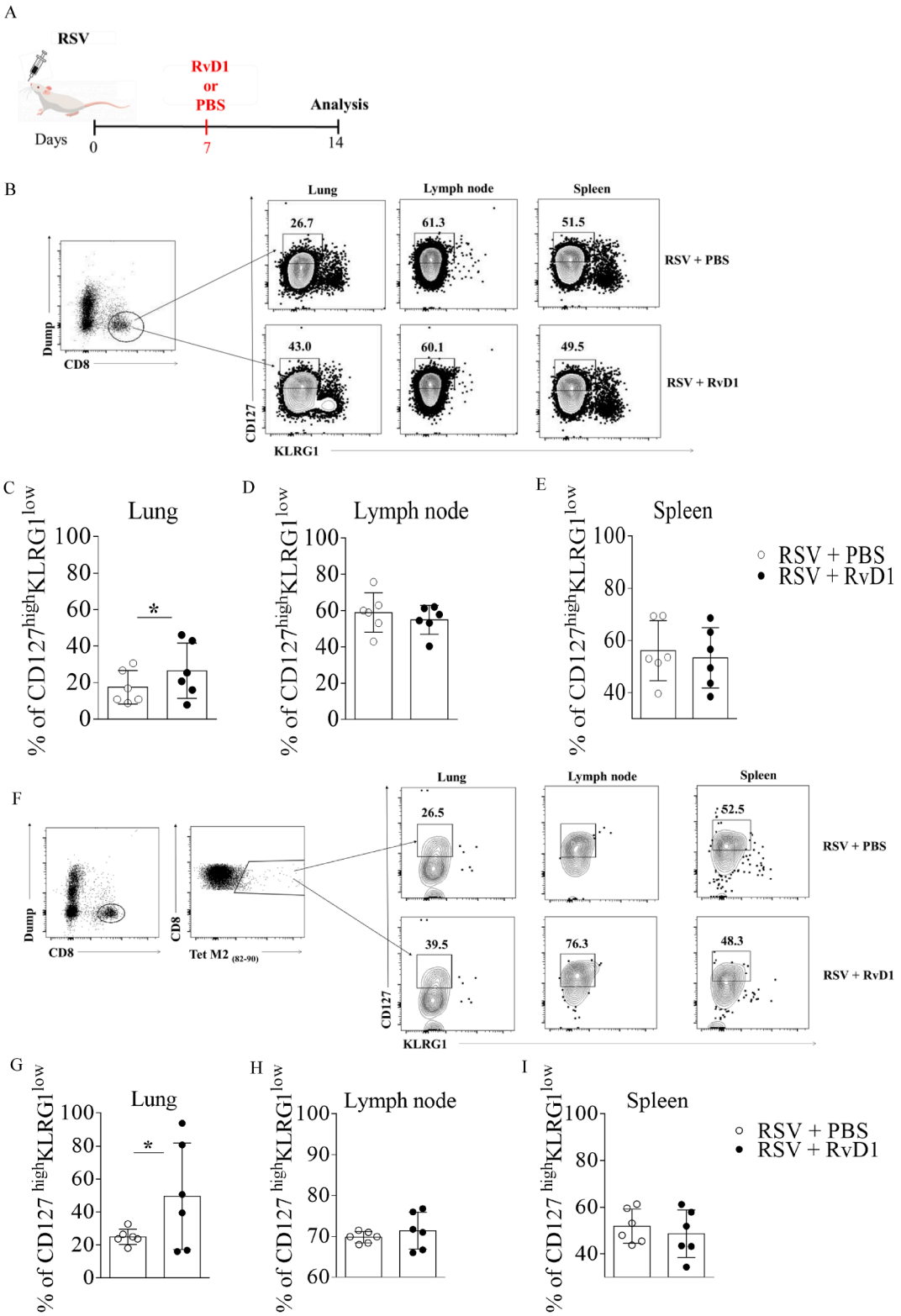

Fig. 4. RvD1 treatment modulates memory CD8+ T cells response in the RSV infection. **(A)** Experimental design of RvD1 treatment on the seventh days of after RSV infection. **(B)** Dot-plot showed the gates of non-specific-RSV memory CD8+ T cells in infected and RvD1 treated mice. **(C-E)** Frequency of non-specific memory CD8+ T cells (CD8 + Dump^{-}CD127^{high}KLRG1^{low}) in the lung, lymph node, and spleen from infected and RvD1 treated mice. **(F)** Dot-plot showed the gates of specific-RSV memory CD8+ T cells in infected and RvD1 treated mice. **(G-I)** Frequency of RSV-specific memory CD8+ T cells (CD8 + Dump^{-}Tet$_{(82\text{-}90)}^{+}$CD127^{high}KLRG1^{low}) in the lung, lymph node, and spleen from infected and RvD1 treated mice. Data were from two independent experiments (n = 6mice/groups). Statistical analysis was determined by the *t* test (* $p < 0.05$, ** $p < 0.01$).

cells in the lymph node and spleen (Fig. 4B, D-F, and H-I). Memory precursors CD8+ T cells of lungs from mice infected and treated were isolated by sorting after 14 days of infection (Fig. 5A-B). These purified cells presented a significantly increased gene expression of cytokines *Il4, Il10, and Infg* (Fig. 5C). The cytokine production of memory CD8 T cells precursors was confirmed by intracellular staining (Fig. 5. D-G).

We next investigated the protective capacity of these memory CD8+ T cells following RSV secondary infection. The infected and treated mice were challenged with the virus after 14 or 49 days of primary infection (Fig. 6A). The analysis was performed after 8 h of reinfection, according to a previously established protocol (Schuurhof et al., 2010; Singleton et al., 2003). RvD1-treated mice present an increased viral load in the lungs after different times of reinfection (Fig. 6B). RvD1 treated-mice during reinfection presented a decreased frequency of memory CD8+ T cells (CD8 + $CD127^{high}KLRG1^{low}$) in the lung after 14 days of primary infection (Fig. 6C-E). We also found that RvD1-treated mice during reinfection showed reduced levels of RSV-specific IgG in the serum after 14 and 49 days of primary infection (Fig. 6F). However, there is no significative difference in the lung inflammatory infiltration (Fig. 6G). We also investigate the RSV-specific memory cells after challenge at 49 days of primary infection (Fig. 7A), and the RvD1 treated mice presented an increase percentage of CD44 + CD69L + RSV-specific cells in the lung (Fig. 7B, C) but no differences were found in other memory cells phenotypes analyzed (Fig. D-L). The IL-12 expression was decreased in the lung (Fig. 7 E). In summary, these data indicated that RvD1 treatment during the resolution phase of RSV primary response modulates the gene expression of memory T cells phenotype, and after challenge with the virus RvD1 contributes to a non-protective response.

4. Discussion

In the present study, we observed that RvD1 can modulate the memory CD8+ T cells both *in vitro* and *in vivo*. However, RvD1 treatment during primary respiratory viral infection results in a non-protective response after secondary infection. RvD1 presents an important role in regulating the resolution phase of inflammation, modulating cells from innate immune response and the tissue repair process (Hsiao et al., 2013; Serhan et al., 2008; Serhan, 2007). The role of RvD1 in CD8+ T cells has been previously demonstrated, RvD1 treatment 30 min before TCR stimulation does not increase human CD8+ T cell proliferation or cell death, but reduces the production of IFN-γ and TNF-α 5 days after culture (Chiurchiù et al., 2016). Here we described that RvD1 treatment before TCR stimulation increases the expansion of human memory CD8+ T cells *in vitro*, but does not interfere in granzyme production in this T cell subtype. RvD1 also is incapable of modulating granzyme production in others types T cells such as regulatory CD4+ T cells (Chiurchiù et al., 2016). Other SPMs have been described to modulated T cell-mediated responses. For example, RvD2 treatment decreases T cell activation and inhibits Th1 phenotype (Mizraji et al., 2018). RvE1 treatment modulates dendritic cells during differentiation inducing T cell apoptosis (Vassiliou et al., 2008). Protectin D1 promotes apoptosis of human T cells (Ariel et al., 2005), and lipoxin A4 and B4 reduce the secretion of TNF-alpha by stimulated human T cells (Ariel et al., 2003).

RvD1 can act through multiple mechanisms, but some have been associated with the PI3K/Akt/mTOR pathway in different models. The efficacy of RvD1 in suppressing myocardial ischemia/reperfusion injury in mice is abrogated by a PI3K inhibitor (Gilbert et al., 2015). The PI3K inhibitor also reduced the efficacy of RvD1 in abolishing inflammation in the lungs in mice models (Wu et al., 2013). RvD1 has been described to phosphorylate AKT and mTOR in macrophages (Gu et al., 2016; Prieto et al., 2015). Our results show that RvD1 decreases IL12p-40 depending on mTOR. This cytokine is secreted by antigen-presenting cells, such as dendritic cells, macrophages, and B cells, and presents proinflammatory activity (Vignali and Kuchroo, 2012). Importantly, IL-12 is involved in the differentiation of memory cells (Raeber et al., 2018). In the moment of T cell activation, the initial presence of IL-12 cytokine during primary infection induces a potent increase of memory cells (Ha et al., 2004), suggesting that early IL-12 signalizing can regulate memory cell generation (Gilbert et al., 2015). However, it has been shown that elevated IL-12 levels impair memory CD8+ T cell response during *Listeria* infection *in vivo* (Joshi et al., 2007). Accordingly, the absence of direct IL-12 signaling to CD8+ T cells is attributed to an improvement in the development of the central memory phenotype during both primary and secondary antigen-specific memory CD8+ T cell responses (Martins Queiroz-Junior et al., 2010; Vignali and Kuchroo, 2012; Gee et al., 2009). Our data indicate that the effect of RvD1 on the expansion of human memory CD8+ T cells *in vitro* might not be related to a direct effect of RvD1 on T cells, but on monocytes and dendritic cells, probably by reducing the production of IL-12 by these cells.

RvD1 treatment 30 min before RSV infection in a murine model decreases the frequency of memory T CD8+ cells precursors in the lung; this data is not consistent with *in vitro* results using human PBMCs, in which RvD1, before TCR engagement, increases the frequency of memory CD8+ T cells. We believe that these differences in RvD1 effects are due to the complexity of an *in vivo* environment during a respiratory viral infection and several stimuli that the CD8+ T cells face, rather than TCR stimulation alone. Nevertheless, when we treated RSV-infected mice with RvD1 after 7 days of infection, we found an increase in the frequency of memory CD8+ T cells in the lung, both specific as well as non-specific to the virus. The treatment of RVD1 after 7 days of infection was in the interface between the expansion and contraction phases of CD8+ T cells (Williams and Bevan, 2007; Harty and Badovinac, 2008; Joshi and Kaech, 2008; Prlic et al., 2007). Additionally, after 7 days of infection, the viral load in the lung of the mice is low, and the mice are recovering from weight loss (Antunes et al., 2019; Gassen et al., 2021; Fazolo et al., 2018). During this period, T cells have already been primed by dendritic cells, and the cytokines milieu can also contribute to the formation of memory CD8+ T cells. Studies suggested that the stimuli received during the early stages of infection promote the generation of effector CD8 T cells, whereas cues at the later stages of infection influence memory differentiation (MA, W., B. MJ., 2004).

This was confirmed by one seminal study that showed the association of the IL-10-IL-21-STAT-3 pathway with memory CD8+ T cells development during LCMV infection. The absence of IL-10 and IL-21 or STAT-3 damages the process of maturation of precursor memory cells to the virus (Cui et al., 2011). Although previous studies have suggested that a resolution of inflammation may contribute to protective memory response, in our model using RvD1 we found divergent results.

Here we described an unfavorable role for RvD1 when administered at the end of primary infection during a secondary response to a respiratory viral infection. The direct effect of RvD1 on RSV infection has not been tested previously, nevertheless, other studies suggested that the use of SPM might be beneficial to reduce lung pathology induced by RSV (Russell and Schwarze, 2014). Mice deficient in 5-lipoxygenase (5-LO), an enzyme required for the SPM lipoxin production, when infected with RSV failed to elicit macrophages with the required phenotypes for the resolution of RSV infection (Shirey et al., 2014). Treatment with lipoxin A4 and resolvin E1 (RvDE1) partially restored the alternatively protective macrophage phenotype in the 5-LO-deficient mice (Shirey et al., 2014). RSV infection increase the levels of leukotrienes, 5-LO (Behera et al., 1998), COX-2, and PGE2 (Walker et al., 2021), and treatment with COX-2 inhibitors decreases lung pathology associated with RSV infection (Richardson et al., 2005). Intranasal administration of the SPM, protectin D1 (PD1) and protectin conjugates in tissue regeneration 1 (PCTR1), reduce viral load during RSV infection (Walker et al., 2021). Also, consumption ômega-3 during pregnancy, the precursor for RvDs production, can protect the offspring children under the age of two from asthmatic symptoms and respiratory infections (Bisgaard et al., 2016). Nevertheless, our results showed that treatment with RvD1 during RSV primary infection contributes to unprotective phenotype for reinfection. We found that RvD1-treated mice had increased viral loads in the lung during reinfection, 14 and 49 days after primary infection. RvD1-treated

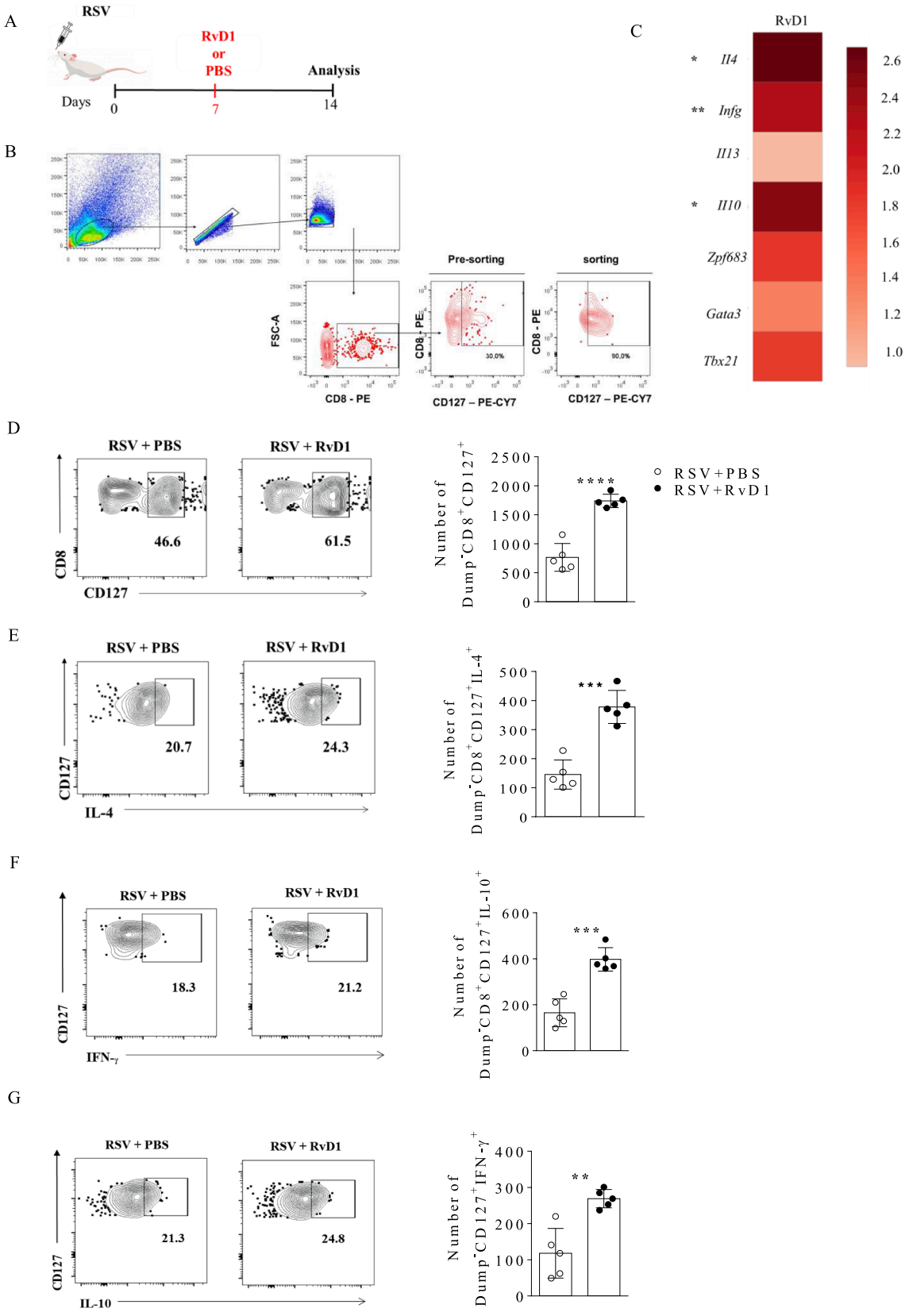

Fig. 5. RvD1 treatment induces the cytokine genes *Il4*, *Il10* and, *Infg* on memory CD8+ T cells in the RSV infection. **(A)** Experimental design of RvD1 treatment on the seventh days of after RSV infection. **(B)** Gate strategy of sorted memory CD8+ T cells (CD8 + $CD127^{high}$) in the lung tissue of mice infected and treated after 14 days of infection. **(C)** Cytokine gene expression *Il4*, *Il10, Infg*, and *Il13*G and gene expression of transcription factors *Gata3*, *Tbx21*, and *Zpf683*. **(D – G)** Intracellular staining of Memory CD8+ T cells producing IL-4, IFN- γ and IL-10. Data are from two independent experiments (n = 6mice/groups). Statistical analysis was determined by the *t* test (* $p < 0.05$, ** $p < 0.01$).

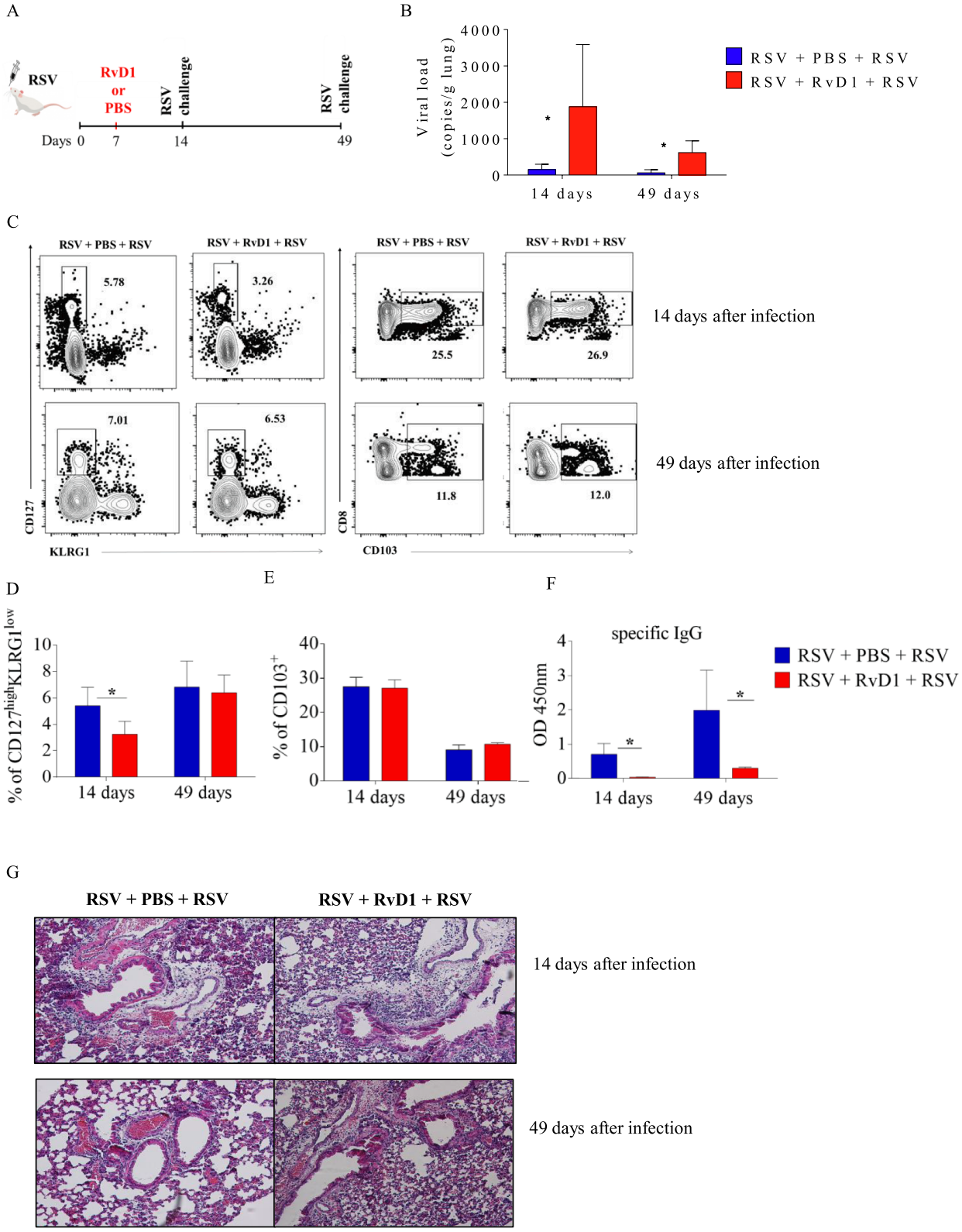

Fig. 6. RvD treatment increases viral load and diminishes the production of virus-specific-IgG in the RSV reinfection after 14 and 49 days of infection. **(A)** Experimental design of RvD1, and RSV reinfection after 14 and 49 days of primary infection. **(B)** Viral load from lung tissue by RT-qPCR (copies of virus/ g lung tissue). **(C)** Dot-plot showing the gate of memory CD8+ T cells in the lung. **(D)** Frequency of memory CD8+ T cell (CD8 + Dump$^-$CD127^{high}KLRG1^{low}). **(E)** Frequency of CD103$^+$CD8+ T cell (CD8 + Dump$^-$CD103$^+$). **(F)** Analysis of virus-specific IgG n the serum. **(G)** Representative images of lung tissue section stained with hematoxylin and eosin (H&E) (n = 3). Data are from two independent experiments (n = 6 mice/groups). Statistical analysis was determined by the *t* test (* $p < 0.05$, ** $p < 0.01$).

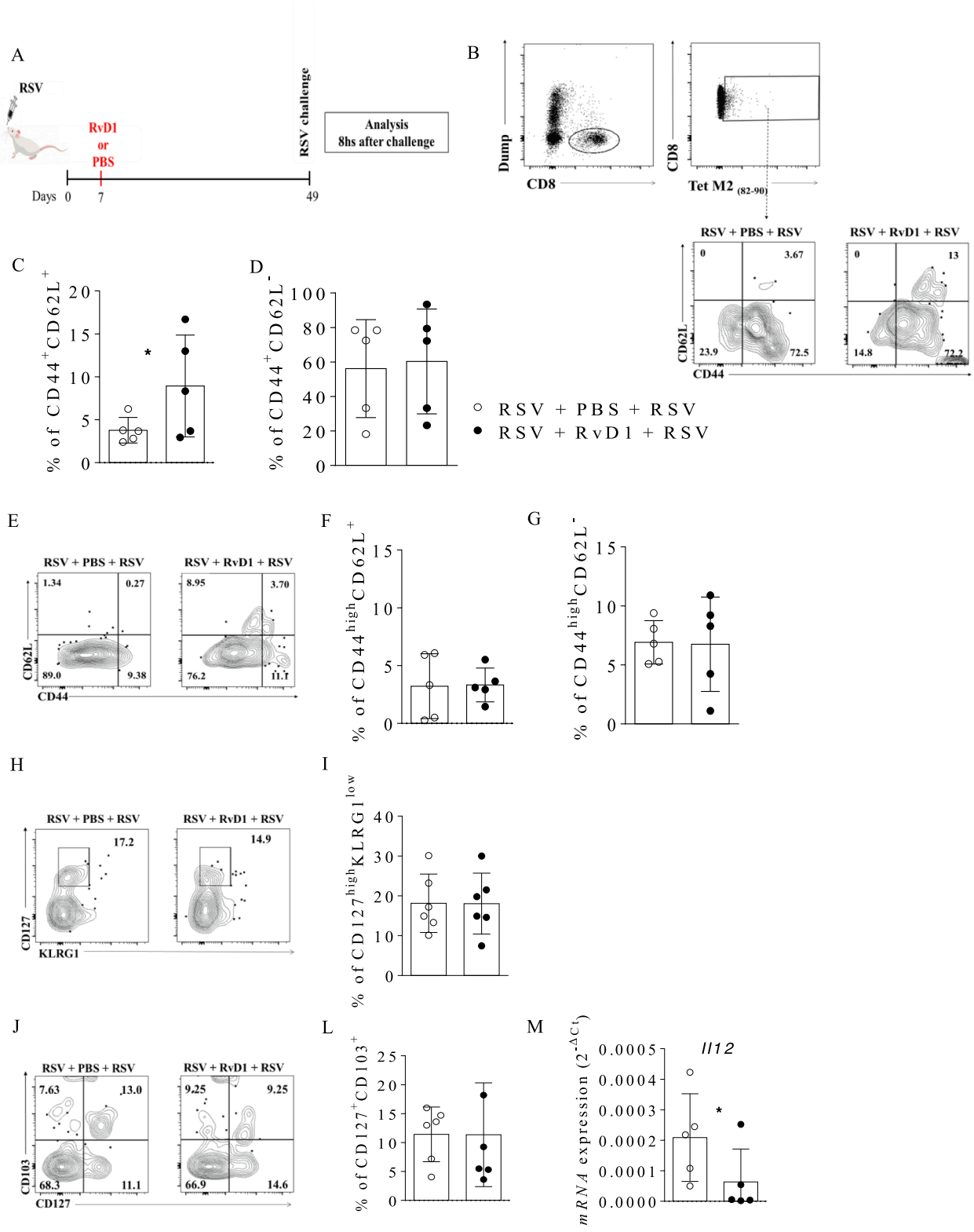

Fig. 7. RvD1increases effector memory cells and decreasing IL-12 expression in the lung after challenge at 49 days of primary infection. **(A)** Experimental design of RvD1, and RSV reinfection after 49 days of primary infection. **(B)** Dot-plot showing the gate of memory CD8+ T cells in the lung. **(C)** Frequency of memory effector CD8+ T cell (CD8 + $Dump^{-}Tet_{(82\text{-}90)}{}^{+}CD44^{+}CD62L^{+}$). **(D)** Frequency of memory center CD8+ T cell (CD8 + $Dump^{-}Tet_{(82\text{-}90)}{}^{+}CD44^{+}CD62L^{-}$). (E) Analysis *Il12* expression in the lung by qPCR. Data are from two independent experiments (n = 6 mice/groups). Statistical analysis was determined by the *t* test (* $p < 0.05$, ** $p < 0.01$).

mice showed an increase percentage of RSV-specific CD44 memory CD8 T cell, but not the other phenotypes of memory cells. Although we found in the lung of RvD1-treated mice a reduced expression of IL-12, this effect is not associated with expansion of memory CD8 T cells.

Our data reveal that memory cells from the lung of RSV-infected and RvD1-treated mice present increased gene expression of *IFN-γ*, *Il-4 e IL-10* after 14 days of infection. Similarly to our findings, a human effector memory CD8+ T cell profile that produce Th2 profile cytokines, express the IL-6Rα receptor, have increased levels of GATA-3 and decreased levels of T-bet and Blimp-1 has been described and in response to RSV infection, these memory cells were able to produce IL-13, IL-5, and IFN-γ *in vitro* (Lee et al., 2014). According, the severity of RSV disease is associated with a CD8+ T profile in the nasal aspirates, these cells produce IL-4 that might play a deleterious role during infection (Siefker et al., 2020). Some studies have demonstrated the importance of CD8+ T cells in control virus load in the lungs after RSV reinfection (Slütter et al., 2013; Channappanavar et al., 2014; Schmidt et al., 2018). Nevertheless, CD8+ T cells were already associated with immunopathology (Graham et al., 1991), and RSV-specific memory CD8+ T cells can also induce a severe immunopathology after reinfection when cells were stimulated in the absence of CD4+ T cells and specific antibodies (Schmidt et al., 2018). Recently, the role of CD8+ T cells in conferring RSV immunopathology was associated with a previous neutrophilic inflammation (Graham et al., 1991; Habibi et al., 2020). Intervention that aims to modulate the CD8+ T cell phenotype decreasing the production of IL-4 might be beneficial to control primary and secondary RSV infection.

We also found that RvD1-treated mice after RSV infection presented decreased levels of specific serum IgG during RSV reinfection. Contrariwise, RvD1 and AT-RvD1 are described to influence B cell differentiation and increase the production of IgG and IgM *in vitro*, but decreasing the secretion of IgE in allergic diseases (Kim et al., 2016; Ramon et al., 2012). In the RSV infection, the antibody response decreases rapidly over time. Low titers of RSV-specific IgG result in weak protection against reinfection (Graham et al., 1991). Also, the absence of B cells does not interfere with the virus elimination but impairs the viral clearance after the reinfection (Graham et al., 1991). Some studies have demonstrated that some phenotypes of CD8+ T cells can influence the production of antibodies from B cells (Valentine and Hoyer, 2019). In our model, we cannot confirm that the non-protective profile found during reinfection in RvD1 treated mice, associated with increased viral load and decreased RSV-specific antibody levels is related to CD8+ T cells memory response. RvD1 was administered systemically, thus we cannot exclude diverse effects of the treatment occurring on different cells.

In conclusion, our results emphasize that effects RvD1 on primary infection have consequences for reinfection, stressing that an accurate inflammation it is important for an effective immune response against RSV. Treatment of RvD1 during primary infection modulates memory CD8+ T cells *in vivo* and induces a non-protective response after RSV reinfection. Our data provide important information regarding a mediator of inflammation resolution and secondary response to a respiratory virus.

5. Grant support

This study was supported by Conselho Nacional de Pesquisa (CNPq) (grant number 477359/2013-2). Deise do Nascimento de Freitas receives PhD scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior – Brasil (CAPES). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior – Brasil (CAPES) – Finance Code 001.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Simopoulos, A.P., 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. J. Am. Coll. Nutr. 21, 495–505.
Bannenberg, G., Serhan, C.N., 2010. Specialized pro-resolving lipid mediators in the inflammatory response: an update. Biochim. Biophys. Acta (BBA) – Mol. Cell Biol. Lipids 1801, 1260–1273.
Schmitz, G., Ecker, J., 2008. The opposing effects of n−3 and n−6 fatty acids. Prog. Lipid Res. 47, 147–155.
Isobe, Y., Arita, M., Iwamoto, R., Urabe, D., Todoroki, H., Masuda, K., Inoue, M., Arai, H., 2013. Stereochemical assignment and anti-inflammatory properties of the omega-3 lipid mediator resolvin E3. J. Biochem. 153, 355–360.
Levy, B.D., Clish, C.B., Schmidt, B., Gronert, K., Serhan, C.N., 2001. Lipid mediator class switching during acute inflammation: signals in resolution. Nat. Immunol. 2, 612–619.
Serhan, C.N., 2004. A search for endogenous mechanisms of anti-inflammation uncovers novel chemical mediators: missing links to resolution. Histochem. Cell Biol. 122, 305–321.
Serhan, C.N., Arita, M., Hong, S., Gotlinger, K., 2004. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. Lipids 39, 1125–1132.
Hong, S., Lu, Y., 2013. Omega-3 fatty acid-derived resolvins and protectins in inflammation resolution and leukocyte functions: targeting novel lipid mediator pathways in mitigation of acute kidney injury. Front. Immunol. 4, 13.
Buckley, C., Gilroy, D., Serhan, C., 2014. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity 40, 315–327.
Gu, Z., G. J. Lamont, R. J. Lamont, S. M. Uriarte, H. Wang, and D. A. Scott. Resolvin D1, resolvin D2 and maresin 1 activate the GSK3b anti-inflammatory axis in TLR4-engaged human monocytes.
Zhang, L., Zhang, X., Wu, P., Li, H., Jin, S., Zhou, X., Li, Y., Ye, D., Chen, B., Wan, J., 2008. BML-111, a lipoxin receptor agonist, modulates the immune response and reduces the severity of collagen-induced arthritis. Inflamm. Res. 57, 157–162.
Poisson, L.M., Suhail, H., Singh, J., Datta, I., Denic, A., Labuzek, K., Hoda, M.N., Shankar, A., Kumar, A., Cerghet, M., Elias, S., Mohney, R.P., Rodriguez, M., Rattan, R., Mangalam, A.K., Giri, S., 2015. Untargeted plasma metabolomics identifies endogenous metabolite with drug-like properties in chronic animal model of multiple sclerosis. J. Biol. Chem. 290, 30697–30712.
Hua, J., Jin, Y., Chen, Y., Inomata, T., Lee, H., Chauhan, S.K., Petasis, N.A., Serhan, C.N., Dana, R., 2014. The resolvin D1 analogue controls maturation of dendritic cells and suppresses alloimmunity in corneal transplantation. Invest. Ophthalmol. Vis. Sci. 55, 5944–5951.
Chiurchiù, V., Leuti, A., Dalli, J., Jacobsson, A., Battistini, L., Maccarrone, M., Serhan, C. N., 2016. Proresolving lipid mediators resolvin D1, resolvin D2, and maresin 1 are critical in modulating T cell responses. Sci. Transl. Med. 8, 353ra111.
Joshi, N.S., Cui, W., Chandele, A., Lee, H.K., Urso, D.R., Hagman, J., Gapin, L., Kaech, S. M., 2007. Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. Immunity 27, 281–295.
Osborne, L. C., and N. Abraham. Regulation of memory T cells by cc cytokines.
Pham, N.-L.L., Badovinac, V.P., Harty, J.T. A Default Pathway of Memory CD8 T Cell Differentiation after Dendritic Cell Immunization Is Deflected by Encounter With Inflammatory Cytokines During Antigen-Driven Proliferation. .
Valbon, S.F., Condotta, S.A., Richer, M.J., 2016. Regulation of effector and memory CD8 + T cell function by inflammatory cytokines. Cytokine 82, 16–23.
Jarjour, N.N., Masopust, D., Jameson, S.C., 2021. T cell memory: understanding COVID-19. Immunity 54, 14–18.
Bont, L., Versteegh, J., Swelsen, W.T.N., Heijnen, C.J., Kavelaars, A., Brus, F., Draaisma, J.M.T., Pekelharing-Berghuis, M., van Diemen-Steenvoorde, R.A.A.M., Kimpen, J.L.L., 2002. Natural reinfection with respiratory syncytial virus does not boost virus-specific T-cell immunity. Pediatr. Res. 52, 363–367.
Ascough, S., Paterson, S., Chiu, C., 2018. Induction and subversion of human protective immunity: contrasting influenza and respiratory syncytial virus. Front. Immunol. 9, 323.
Pham, N.-L.-L., Badovinac, V.P., Harty, J.T., 2011. Differential role of "signal 3" inflammatory cytokines in regulating CD8 T cell expansion and differentiation in vivo. Front. Immunol. 2, 4.
Richer, M.J., Nolz, J.C., Harty, J.T. 2012. Pathogen-specific inflammatory milieux tune the antigen sensitivity of CD8 + T cells by enhancing T cell receptor signaling.
Pearce E.L., Shen, H. 2007. Generation of CD8 T cell memory is regulated by IL-12. *J. Immunol. (Baltimore, Md. : 1950)* 179: 2074–81.
Laidlaw, B.J., Cui, W., Amezquita, R.A., Gray, S.M., Guan, T., Lu, Y., Kobayashi, Y., Flavell, R.A., Kleinstein, S.H., Craft, J., Kaech, S.M., 2015. Production of IL-10 by CD4+ regulatory T cells during the resolution of infection promotes the maturation of memory CD8+ T cells. Nat. Immunol. 16, 871–879.
Geginat, J., Lanzavecchia, A., Sallusto, F., 2003. Proliferation and differentiation potential of human CD8+ memory T-cell subsets in response to antigen or homeostatic cytokines. Blood 101, 4260–4266.
H, P.-G., A.-C. D, F.-V. H, M. JJ, L.-G. JS. 2005. Effector, memory and naïve CD8+ T cells in peripheral blood and pleural effusion from lung adenocarcinoma patients. *Lung Cancer (Amsterdam, Netherlands)* 47: 361–371.
Araki, K., Youngblood, B., Ahmed, R. The role of mTOR in memory CD8 + T-cell differentiation.

Pompura, S.L., Dominguez-Villar, M., 2018. The PI3K/AKT signaling pathway in regulatory T-cell development, stability, and function. J. Leukoc. Biol. 103, 1065–1076.
Raeber, M.E., Zurbuchen, Y., Impellizzieri, D., Boyman, O., 2018. The role of cytokines in T-cell memory in health and disease. Immunol. Rev. 283, 176–193.
Martins Queiroz-Junior, C., M. J. Barbosa Silva, J. Dias Corrêa, M. Fernandes, M. Madeira, T. P. Garlet, G. P. Garlet, F. Q. Cunha, M. Martins Teixeira, and T. Aparecida Da Silva. 2010. A controversial role for IL-12 in immune response and bone resorption at apical periodontal sites. *Clin. Develop. Immunol.* 2010.
Vignali, D.A.A., Kuchroo, V.K., 2012. IL-12 family cytokines: immunological playmakers. Nat. Immunol. 13, 722–728.
Gee, K., Guzzo, C., Mat, N.F.C., Ma, W., Kumar, A., 2009. The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. Inflam. Allergy - Drug Targets 8, 40–52.
Schuurhof, A., L. Bont, J. L. A. Pennings, H. M. Hodemaekers, P. W. Wester, A. Buisman, L. C. G. H. de Rond, M. N. Widjojoatmodjo, W. Luytjes, J. L. L. Kimpen, R. Janssen. 2010. Gene expression differences in lungs of mice during secondary immune responses to respiratory syncytial virus infection. *J. Virol.* 84: 9584–9594.
Singleton, R., Etchart, N., Hou, S., Hyland, L., 2003. Inability to evoke a long-lasting protective immune response to respiratory syncytial virus infection in mice correlates with ineffective nasal antibody responses. J. Virol. 77, 11303–11311.
Hsiao, H.-M., Sapinoro, R.E., Thatcher, T.H., Croasdell, A., Levy, E.P., Fulton, R.A., Olsen, K.C., Pollock, S.J., Serhan, C.N., Phipps, R.P., Sime, P.J., Yildirim, A.Ö., 2013. A novel anti-inflammatory and pro-resolving role for resolvin D1 in acute cigarette smoke-induced lung inflammation. PLoS ONE.
Serhan, C.N., Chiang, N., Van Dyke, T.E., 2008. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat. Rev. Immunol. 8, 349–361.
Serhan, C. N. 2007. Resolution Phase of Inflammation: Novel Endogenous Anti-Inflammatory and Proresolving Lipid Mediators and Pathways.
Mizraji, G., Heyman, O., Van Dyke, T.E., Wilensky, A., 2018. Resolvin D2 restrains Th1 immunity and prevents alveolar bone loss in murine periodontitis. Front. Immunol. 9, 785.
Vassiliou, E.K., Kesler, O.M., Tadros, J.H., Ganea, D., 2008. Bone Marrow-derived dendritic cells generated in the presence of resolvin E1 induce apoptosis of activated CD4 + T cells. J. Immunol. 181, 4534–4544.
Ariel, A., Li, P.L., Wang, W., Tang, W.X., Fredman, G., Hong, S., Gotlinger, K.H., Serhan, C.N., 2005. The docosatriene protectin D1 is produced by TH2 skewing promotes human T cell via lipid raft clustering. J. Biol. Chem. 280, 43079–43086.
Ariel, A., Chiang, N., Arita, M., Petasis, N.A., Serhan, C.N., 2003. Aspirin-triggered lipoxin A 4 and B 4 analogs block extracellular signal-regulated kinase-dependent TNF-α secretion from human T cells. J. Immunol. 170, 6266–6272.
Gilbert, K., Bernier, J., Bourque-Riel, V., Malick, M., Rousseau, G., 2015. Resolvin D1 reduces infarct size through a phosphoinositide 3-kinase/protein kinase B mechanism. J. Cardiovasc. Pharmacol. 66, 72–79.
Wu, D., S. Zheng, W. Li, L. Yang, Y. Liu, X. Zheng, Y. Yang, L. Yang, Q. Wang, F. G. Smith, S. Jin. 2013. Novel Biphasic Role of Resolvin D1 on expression of cyclooxygenase-2 in lipopolysaccharide-stimulated lung fibroblasts is partly through PI3K/AKT and ERK2 pathways. *Mediators of Inflammation* 11.
Prieto, P., Rosales-Mendoza, C.E., Terrón, V., Toledano, V., Cuadrado, A., López-Collazo, E., Bannenberg, G., Martín-Sanz, P., Fernández-Velasco, M., Boscá, L., 2015. Activation of autophagy in macrophages by pro-resolving lipid mediators. Autophagy 11, 1729–1744.
Ha, S.-J., Sung, Y.-C., Chang, J., Cho, J.-H., Lee, S.-W., Choi, S.-Y., 2004. and memory cells cells and increases generation of effector stimulation changes properties of CD8 T IL-12 priming during in vitro antigenic. J Immunol References 172, 2818–2826.
Williams, M.A., Bevan, M.J., 2007. Effector and memory CTL differentiation. Annu. Rev. Immunol. 25, 171–192.
Harty, J.T., Badovinac, V.P., 2008. Shaping and reshaping CD8+ T-cell memory. Nat. Rev. Immunol. 8, 107–119.
Joshi, N.S., Kaech, S.M., 2008. Effector CD8 T cell development: a balancing act between memory cell potential and terminal differentiation. J. Immunol. 180, 1309–1315.
Prlic, M., Williams, M.A., Bevan, M.J., 2007. Requirements for CD8 T-cell priming, memory generation and maintenance. Curr. Opin. Immunol. 19, 315–319.
Antunes, K.H., Fachi, J.L., de Paula, R., da Silva, E.F., Pral, L.P., dos Santos, A.Á., Dias, G. B.M., Vargas, J.E., Puga, R., Mayer, F.Q., Maito, F., Zárate-Bladés, C.R., Ajami, N.J., Sant'Ana, M.R., Candreva, T., Rodrigues, H.G., Schmiele, M., Silva Clerici, M.T.P., Proença-Modena, J.L., Vieira, A.T., Mackay, C.R., Mansur, D., Caballero, M.T., Marzec, J., Li, J., Wang, X., Bell, D., Polack, F.P., Kleeberger, S.R., Stein, R.T., Vinolo, M.A.R., de Souza, A.P.D., 2019. Microbiota-derived acetate protects against respiratory syncytial virus infection through a GPR43-type 1 interferon response. Nature Commun. 10.
Gassen, R.B., Fazolo, T., Nascimento de Freitas, D., Borges, T.J., Lima, K., Antunes, G.L., Maito, F., Bueno Mendes, D.A.G., Báfica, A., Rodrigues, L.C., Stein, R., Duarte de Souza, A.P., Bonorino, C., 2021. IL-21 treatment recovers follicular helper T cells and neutralizing antibody production in respiratory syncytial virus infection. Immunol. Cell Biol. 99, 309–322.
Fazolo, T., Gassen, R.B., de Freitas, D.N., Borges, T.J., Rigo, M.M., da Silva, R.D., Maito, F., Cunha, A., Gasparin Bueno Mendes, D.A., Báfica, A., Vargas, J.E., de Souza, A.P.D., Bonorino, C., 2018. Vaccination with RSV M209–223 peptide promotes a protective immune response associated with reduced pulmonary inflammation. Antiviral Res. 157, 102–110.
MA, W., B. MJ. 2004. Shortening the infectious period does not alter expansion of CD8 T cells but diminishes their capacity to differentiate into memory cells. *J. Immunol. (Baltimore, Md. : 1950)* 173: 6694–6702.
Cui, W., Y. Liu, J. S. Weinstein, J. Craft, S. M. Kaech. 2011. An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8+ T cells. *Immunity* 35: 792–805.
Russell, C.D., Schwarze, J., 2014. The role of pro-resolution lipid mediators in infectious disease. Immunology 141, 166–173.
Shirey, K.A., Lai, W., Pletneva, L.M., Karp, C.L., Divanovic, S., Blanco, J.C.G., Vogel, S.N., 2014. Role of the lipoxygenase pathway in RSV-induced alternatively activated macrophages leading to resolution of lung pathology. Mucosal Immunol. 7, 549–557.
Behera, A.K., Kumar, M., Matsuse, H., Lockey, R.F., Mohapatra, S.S., 1998. Respiratory syncytial virus induces the expression of 5-lipoxygenase and endothelin-1 in bronchial epithelial cells. Biochem. Biophys. Res. Commun. 251, 704–709.
Richardson, J.Y., Ottolini, M.G., Pletneva, L., Boukhvalova, M., Zhang, S., Vogel, S.N., Prince, G.A., Blanco, J.C.G., 2005. Respiratory syncytial virus (RSV) infection induces cyclooxygenase 2: a potential target for RSV therapy. J. Immunol. 174, 4356–4364.
Walker, K.H., Krishnamoorthy, N., Brüggemann, T.R., Shay, A.E., Serhan, C.N., Levy, B. D., 2021. Protectins PCTR1 and PD1 reduce viral load and lung inflammation during respiratory syncytial virus infection in mice. Front. Immunol. 12, 704427.
Bisgaard, H., Stokholm, J., Chawes, B.L., Vissing, N.H., Bjarnadóttir, E., Schoos, A.-M., Wolsk, H.M., Pedersen, T.M., Vinding, R.K., Thorsteinsdóttir, S., Følsgaard, N.V., Fink, N.R., Thorsen, J., Pedersen, A.G., Waage, J., Rasmussen, M.A., Stark, K.D., Olsen, S.F., Bønnelykke, K., 2016. Fish oil-derived fatty acids in pregnancy and wheeze and asthma in offspring. N. Engl. J. Med. 375, 2530–2539.
Lee, N., You, S., Shin, M.S., Lee, W.-W., Kang, K.S., Kim, S.H., Kim, W.-U., Homer, R.J., Kang, M.-J., Montgomery, R.R., Dela Cruz, C.S., Shaw, A.C., Lee, P.J., Chupp, G.L., Hwang, D., Kang, I., 2014. IL-6 receptor α defines effector memory CD8+ T cells producing Th2 cytokines and expanding in asthma. Am. J. Respir. Crit. Care Med. 190, 1383–1394.
Siefker, D. T., L. Vu, D. You, A. McBride, R. Taylor, T. L. Jones, J. DeVincenzo, S. A. Cormier. 2020. Respiratory syncytial virus disease severity is associated with distinct CD8+ T-cell profiles. *https://doi.org/10.1164/rccm.201903-0588OC* 201: 325–334.
Slütter, B., L. L. Pewe, S. M. Kaech, J. T. Harty. 2013. Lung airway-surveilling CXCR3(hi) memory CD8(+) T cells are critical for protection against influenza A virus. *Immunity* 39: 939–48.
Channappanavar, R., Fett, C., Zhao, J., Meyerholz, D.K., Perlman, S., Sandri-Goldin, R. M., 2014. Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome coronavirus infection. J. Virol. 88, 11034–11044.
Schmidt, M. E., C. J. Knudson, S. M. Hartwig, L. L. Pewe, D. K. Meyerholz, R. A. Langlois, J. T. Harty, S. M. Varga. 2018. Memory CD8 T cells mediate severe immunopathology following respiratory syncytial virus infection. *PLoS Pathogens* 14.
Graham, B.S., Bunton, L.A., Wright, P.F., Karzon, D.T., 1991. Role of T lymphocyte subsets in the pathogenesis of primary infection and rechallenge with respiratory syncytial virus in mice. J. Clin. Invest. 88, 1026–1033.
Habibi, M.S., Thwaites, R.S., Chang, M., Jozwik, A., Paras, A., Kirsebom, F., Varese, A., Owen, A., Cuthbertson, L., James, P., Tunstall, T., Nickle, D., Hansel, T.T., Moffatt, M.F., Johansson, C., Chiu, C., Openshaw, P.J.M., 2020. Neutrophilic inflammation in the respiratory mucosa predisposes to RSV infection. Science 370.
Kim, N., Ramon, S., Thatcher, T.H., Woeller, C.F., Sime, P.J., Phipps, R.P., 2016. Specialized proresolving mediators (SPMs) inhibit human B-cell IgE production. Eur. J. Immunol. 46, 81–91.
Ramon, S., Gao, F., Serhan, C.N., Phipps, R.P., 2012. Specialized proresolving mediators enhance human b cell differentiation to antibody-secreting cells. J. Immunol. 189, 1036–1042.
Graham, B. S., L. A. Bunton, P. F. Wright, D. T. Karzon. 1991. Reinfection of mice with respiratory syncytial virus.[Erratum appears in J Med Virol 1991; 35(4):307].
Graham, B.S., Bunton, L.A., Rowland, J., Wright, P.F., Karzon, D.T., 1991. Respiratory syncytial virus infection in anti-mu-treated mice. J. Virol. 65, 4936–4942.
Valentine, K. M., K. K. Hoyer. 2019. CXCR5+ CD8 T Cells: Protective or Pathogenic? *Front. Immunol.* 10.