IL-1 receptor 1 signaling shapes the development of viral antigen-specific CD4$^+$ T cell responses following COVID-19 mRNA vaccination

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Summary

Background The innate immune cytokine interleukin (IL)-1 can affect T cell immunity, a critical factor in host defense. In a previous study, we identified a subset of human CD4$^+$ T cells which express IL-1 receptor 1 (IL-1R1). However, the expression of such receptor by viral antigen-specific CD4$^+$ T cells and its biological implication remain largely unexplored. This led us to investigate the implication of IL-1R1 in the development of viral antigen-specific CD4$^+$ T cell responses in humans, including healthy individuals and patients with primary antibody deficiency (PAD), and animals.

Methods We characterized CD4$^+$ T cells specific for SARS-CoV-2 spike (S) protein, influenza virus, and cytomegalovirus utilizing multiplexed single cell RNA-seq, mass cytometry and flow cytometry followed by an animal study.

Findings In healthy individuals, CD4$^+$ T cells specific for viral antigens, including S protein, highly expressed IL-1R1. IL-1β promoted interferon (IFN)-γ expression by S protein-stimulated CD4$^+$ T cells, supporting the functional implication of IL-1R1. Following the 2nd dose of COVID-19 mRNA vaccines, S protein-specific CD4$^+$ T cells with high levels of IL-1R1 increased, likely reflecting repetitive antigenic stimulation. The expression levels of IL-1R1 by such cells correlated with the development of serum anti-S protein IgG antibody. A similar finding of increased expression of IL-1R1 by S protein-specific CD4$^+$ T cells was also observed in patients with PAD following COVID-19 mRNA vaccination although the expression levels of IL-1R1 by such cells did not correlate with the levels of serum anti-S protein IgG antibody. In mice immunized with COVID-19 mRNA vaccine, neutralizing IL-1R1 decreased IFN-γ expression by S protein-specific CD4$^+$ T cells and the development of anti-S protein IgG antibody.

Interpretation Our results demonstrate the significance of IL-1R1 expression in CD4$^+$ T cells for the development of viral antigen-specific CD4$^+$ T cell responses, contributing to humoral immunity. This provides an insight into the regulation of adaptive immune responses to viruses via the IL-1 and IL-1R1 interface.

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Research in context

Evidence before this study

$CD4^+$ T cells play a critical role in host defense by helping other immune cells, such as B cells, through secreting cytokines and expressing co-stimulatory molecules. The development of effector $CD4^+$ T cells is dependent on the cytokine milieu, and the responses to such cytokines can be determined by the cytokine receptors expressed on T cells. We previously discovered two distinct subsets of human $CD4^+$ T cells which expressed high and low levels of IL-1 receptor 1 (IL-1R1) with the differential responses to IL-1β. Also, decreased cytokine production was found in mice deficient of IL-1R. A recent study found IL-1β production by human peripheral blood mononuclear cells incubated with lipid-formulated RNA vaccines, implying a potent role of IL-1β in the development of T cell immune responses to mRNA vaccines. Although these findings support the critical role of IL-1 and its receptor interaction in the development of T cell immune responses, little is known about the implication of IL-1R1 expression by $CD4^+$ T cells in the development of $CD4^+$ T cell responses specific to pathogens, especially viral antigens, in the context of repetitive immune stimulations.

Added value of this study

Here, we discovered that viral antigen-specific $CD4^+$ T cells, including those specific for SARS-CoV-2 spike (S) protein, express high levels of IL-1R1 in humans using high-dimensional single cell analyses. Repetitive immunization with COVID-19 mRNA vaccines led to the expansion of S protein-specific $CD4^+$ T cells expressing IL-1R1. Such cytokine receptor expression correlated with anti-S protein IgG levels in healthy subjects but not in patients with primary antibody deficiency (PAD). Additionally, triggering IL-1R1 with IL-1β or blocking IL-1R1 significantly modified $CD4^+$ T cell responses to S protein in human *ex vivo* and murine *in vivo* studies, supporting the role of IL-1R1 and its interaction with IL-1 in determining $CD4^+$ T cell responses to viral antigens such as the S protein.

Implications of all the available evidence

The results of our study offer an insight into the significance of IL-1R1 expression on $CD4^+$ T cells and its interface with IL-1 in promoting viral antigen-specific $CD4^+$ T cell responses, especially in the setting of vaccination. These findings raise the possible consideration of an approach to enhance the efficacy of vaccines, including both mRNA-based and conventional ones, by modifying IL-1 production and its receptor system.

Introduction

The full activation of naïve T cells with proliferation and differentiation into effector cells require three signals via T cell receptor triggering by antigenic peptide-major histocompatibility complex (MHC) complex, co-stimulatory molecules, and cytokines. The cytokines involved in this process are produced largely by innate immune cells such as dendritic cells (DCs), monocytes, and macrophages. For instance, IL-12 is well known to promote $CD4^+$ T helper (Th) cell differentiation with IFN-γ production while IL-23 can enhance IL-17 production by $CD4^+$ T cells.[1] IL-1, including IL-1α and IL-1β, also can affect the production of cytokines including IL-17 and IFN-γ from $CD4^+$ T cells in both humans and mice.[2,3] In addition to naïve T cells, such cytokines can have similar effects on memory T cells. IL-1β promoted IL-17 and IFN-γ production from human memory $CD4^+$ T cells[3–5] while IL-1 permitted effector cytokine production by pre-committed Th1 (IFN-γ), Th2, and Th17 cells in mice.[6]

Distinct cytokine receptor systems exist to recognize groups of cytokines, and such cytokine receptors are often utilized in categorizing different groups of cytokines.[7] Although the levels of cytokines at the site of T cell activation are an important factor in governing the effect of cytokines on T cells, the expression levels of cytokine receptors by T cells can be diverse serving as a determinant of T cell responses to cytokines. Previously, we reported that human naïve and memory $CD4^+$ T cells had two distinct cell subsets which express high and low levels of IL-1 receptor 1 as well as the differential effects of IL-1β on the two cell subsets as determined by IL-17 production.[3] Also, mice deficient of IL-1R had reduced production of cytokines including IL-17, IL-13 and IFN-γ up on TCR triggering with anti-CD3 antibodies.[6]

Data from both human and animal studies indicate the implications of IL-1 and IL-1R1 in regulating cytokine production from $CD4^+$ T cells, especially in the presence of mitogens like anti-CD3 antibodies.[3,5,6,8] However, it is largely unknown about the IL-1R1 expression by $CD4^+$ T cells that are activated by pathogens, especially viral antigens in the context of repetitive stimulation, and the biological implication of such receptor interface with IL-1 in determining effector function of $CD4^+$ T cells. Here we have addressed these questions, which are clinically important given the position of $CD4^+$ T cells in host defense, by characterizing

$CD4^+$ T cells specific for viral antigens including SARS-CoV-2 spike (S) protein in healthy individuals, especially in the setting of COVID-19 mRNA vaccination, utilizing high-dimensional single cell analyses. These findings were furthered by analyzing the same T cells in patients with primary antibody deficiency (PAD) as well as by interrupting the interface of IL-1 and IL-1R1 in animals that received COVID-19 mRNA vaccine. The results of our study demonstrated upregulation of IL-1R1 expression on S protein-specific $CD4^+$ T cells in healthy subjects and patients with PAD. The manipulation of this receptor through cytokine or neutralizing antibody treatment significantly affected the S protein-specific $CD4^+$ T cell responses as determined by cytokine expression in human *in vitro* and murine *in vivo* studies. The results of our study demonstrated the role of IL-1R1 and its interface with IL-1 in shaping $CD4^+$ T cell responses to viral antigens such as S protein.

Methods

Human subjects

Eleven healthy adult subjects (mean age ± standard deviation, 40.8 years ±6.7, 6 males and 5 females) who were going to receive COVID-19 mRNA vaccine were recruited between December 2020 and May 2021. Healthy individuals were those who were not taking immunosuppressive drugs and did not have a disease potentially affecting the immune system including infection, cancer, asthma, immunodeficiency, autoimmunity and diabetes.[3] Healthy subjects received the first and second doses of COVID-19 mRNA vaccine (Moderna vaccine, n = 8 and Pfizer-BioNTech vaccine, n = 3). Twelve patients with PADs (mean age ± standard deviation, 45.1 years ±22.5, 3 males and 9 females) were recruited from Yale Immunodeficiency clinics (Supplementary Table S1 for detailed clinical characteristics). These patients include 4 patients with CVID, 3 patients with IgG deficiency, 1 patient with selective antibody deficiency, and 3 patients with IgG subclass 2 deficiency. Patients with PADs were divided into two groups including CVID and other PADs including the latter three conditions as previously done.[9] Patients with PADs received the first and second doses of COVID-19 mRNA vaccine (Moderna vaccine, n = 6 and Pfizer-BioNTech vaccine, n = 6). After obtaining informed consent, peripheral blood samples were obtained at three time points: prior to vaccination and 3–4 weeks after the first and second doses of the Pfizer-BioNTech or Moderna COVID-19 mRNA vaccine. None of the study subjects had SARS-CoV-2 infection at the three time points of blood sampling as determined by serum anti-nucleocapsid IgG antibody ELISA.

Ethics

The study was reviewed and approved by the Yale University Institutional Review Board (ID 2000022963). Prior to the study, written informed consent was obtained from all participants. The animal protocol (ID 2022-10929) was approved by the Institutional Animal Care and Use Committee of Yale University.

Mice and immunization

C57BL/6 mice (8 weeks old) were purchased from the Jackson Laboratory and maintained under pathogen-free conditions in animal facility at Yale University. Discarded remnant mRNA vaccine was used according to the manufacturer's guideline for storage and dose intervals. Mice were injected intramuscularly with 50 µl of BNT162b2 COVID-19 mRNA vaccine (0.05 µg per mouse) in a three-week interval. For neutralizing IL-1R1, the immunized mice were administered intraperitoneally with 100 µl of anti-IL-1R1 neutralizing antibody (20 µg per mouse, R&D Systems, Minneapolis, MN) at 1 day before, on the day, and 1 day after the 1st and 2nd doses of immunization, respectively. One week after the 2nd dose immunization, the mice were sacrificed for further analysis. The animal protocol was approved by the Institutional Animal Care and Use Committee of Yale University.

Cell isolation

Blood was collected and centrifuged at 1800 rpm for 10 min. The supernatant was removed. PBMCs were isolated from blood (diluted with PBS at 1:1 ratio) by density gradient centrifugation using Ficoll-Paque™ PREMIUM sterile solution (Cytiva, Marlborough, MA).[9] Red blood cells were lysed with RBC lysis buffer (Invitrogen, Waltham, MA).

Flow cytometry, cell stimulation and intracellular cytokine analysis

Purified PBMCs (5×10^5 cells/well) were incubated for 3 days in round-bottom plates under the following conditions: SARS-CoV-2 spike peptides (S peptides, Miltenyi, Auburn, CA) and anti-CD28/49d antibodies (BD Biosciences, Franklin Lakes, NJ), 5 or 10 ng/ml of human recombinant IL-1β (R&D Systems, Minneapolis, MN) only, and combinations of the S peptides and anti-CD28/49d with 5 or 10 ng/ml of IL-1β.[3,9] Incubated cells were washed in the plates and additionally incubated for 6 h with S peptides in the presence of Golgiplug (last 4 h). For IL-1R2 neutralization, anti-IL-1R2 neutralizing antibody (20 µg/ml, Invitrogen) was pre-incubated for 30 min (min) with PBMCs prior to S peptide and co-stimulatory molecule stimulation. For surface staining and intracellular cytokine analysis, after stimulation, live/dead aqua dye (Invitrogen) was added to exclude dead cells and stained with antibodies to BUV395-CD3e (SK7), BV711–CD4 (SK3), Amcyan-CD8 (SK1), PE–Cy5–CD45RA (HI100), PE-Cy7-CCR7 (3D12), FITC-CD134 (Ber-ACT35), APC-CD137 (4B4-1), PE-IL-1R1 (polyclonal) and Alexa Fluor 700-IL-1R2 (34,141). The cells were fixed with BD cytofix/cytoperm for 20 min,

washed, and stained for 30 min with antibodies to BV605-IFN-γ (B27). For mice analysis, isolated splenocytes (1×10^6 cells/well) were incubated overnight in round-bottom plates, either with or without SARS-CoV2 spike peptides in the presence of brefeldin A. After the incubation, cells were stained with antibodies to PE-Cy5-CD3e (145-2C11), Alexa Fluor 700-CD4 (RM4-5), and BUV395-CD44 (IM77) followed by fixation and permeabilization with BD cytofix/cytoperm and staining with antibodies to FITC-IFN-γ (XMG1.2), PE-Cy7-IL-2 (JES6-5H4), and BV605-TNF-α (MP6-XT22). Stained cells were analyzed for $CD4^+$ T cells expressing cytokines using an LSRII flow cytometer®. Flow cytometric data were analyzed by FlowJo software (FlowJo, Ashland, OR).

Multiplexed single cell RNA-seq analysis

PBMCs from healthy subjects were incubated for 18 h in the presence or absence of S peptides, influenza virus (Flu) (Afluria® Quadrivalent 2020–2021, Seqirus, Parkville, Australia) or CMV (Enzo Life Sciences, Farmingdale, NY) lysate antigens with anti-CD28/49d antibodies. The incubated cells were stained with antibodies to CD16, CD4, CD45RA, CCR7, 4-1BB and OX40. Based on OX40 and 4-1BB expression, stained cells from each incubation condition were sorted into different memory ($CD45RA^-CCR7^{+/-}$) $CD4^+$ T cell subsets using a FACSAria®. These subsets were S peptides-incubated $OX40^-4\text{-}1BB^-$, $OX40^-4\text{-}1BB^+$, $OX40^+4\text{-}1BB^-$, and $OX40^+4\text{-}1BB^+$ cells, Flu lysate-incubated $OX40^+4\text{-}1BB^+$ cells, CMV lysate-incubated $OX40^+4\text{-}1BB^+$ cells, and control (no antigenic stimulation) incubated $OX40^-4\text{-}1BB^-$ cells (note: no significant $OX40^+4\text{-}1BB^+$ cells were present in the control). For multiplexed single cell RNA-seq, sorted cells were incubated for 30 min with cell hashtag antibodies (BioLegend, San Diego, CA) conjugated to a unique barcode sequence and applied to a 10× Genomics platform at Yale Center for Genome Analysis (YCGA). R-based Seurat package (Version 4) was used to process the data matrix and analyze multiplexed (hash tagged) data. Cells with singlet hashtag were selected and filtered with nFeature RNA >200, nCount RNA <5000, and percent.mt <5 for further analyses. UMAP analysis was performed to visualize cells in a 2-D space using principal component analysis (PCA) and FindClusters command in the Seurat package. To identify DEGs in each of the clusters relative to the rest of the cells in the analysis, the function FindAllMarkers was used. DEGs were filtered using a minimum fold change >1.5 and a maximum false discovery rate (FDR) <0.05. Gene Set Enrichment Analysis (GSEA) was done using the R-based GSEA package.

CyTOF analysis

All CyTOF antibodies (Supplementary Table S2) were commercially available as a metal-tagged or CyTOF ready form (Fluidigm, South San Francisco, CA). For cross-sectional analysis, PBMCs were incubated for overnight with S peptides, Flu or CMV antigens in the presence of anti-CD28/CD49d antibodies. For longitudinal analysis following COVID-19 vaccination, PBMCs were incubated for overnight with or without S peptides and anti-CD28/CD49d antibodies. Incubated cells were stained with Cisplatin (viability) staining to exclude dead cells. Cells were resuspended with MaxPar Water containing EQ Four Element Calibration Beads and acquired on a CyTOF system Helios (Fluidigm). All FCS files were normalized with bead standards and analyzed using the analytic tool CYT and FlowJo software. The FCS files were transformed using an inverse hyperbolic sine (arcsinh) function with a cofactor of 5 and pre-gated manually to exclude EQ beads, cell debris, cell doublets and dead cells. For our clustering analysis, we pre-gated $CD3^+CD4^+$ memory ($CD45RA^-CCR7^{+/-}$) T cells based on the expression of CD45RA and CCR7. These memory $CD4^+$ T cells were further gated by the expression of OX40 and 4-1BB to identify $OX40^-4\text{-}1BB^-$, $OX40^+4\text{-}1BB^-$, $OX40^-4\text{-}1BB^+$, and $OX40^+4\text{-}1BB^+$ in cells incubated in different conditions. Data were further analyzed using t-SNE, PhenoGraph, and metaclustering.[10,11]

Statistical analysis

The data were assessed for normal distribution using the Shapiro–Wilk test and for the assumption of homogeneity of variance through Levene's test. Non-parametric analyses were conducted when the assumption of normality or homogeneity of variance was violated. Statistical analysis was performed using the one-way ANOVA with Dunnett's post hoc analysis, the Wilcoxon matched-pairs signed rank test, the Signed test, the paired *t*-test, the unpaired *t*-test or Pearson correlation as appropriate based on the results of assessing normality and a symmetrical distribution of differences using GraphPad Prism 10.01 or SAS. The sample size calculations for the experiments of assessing different levels of IL-1R1 expression by $CD4^+$ T cell subsets incubated with S protein and the effect of IL-1β on cytokine expression by S protein-specific $CD4^+$ T cells were done based on our previous work which assessed IL-1R1 expression by $CD4^+$ T cells and the effects of IL-1β on IL-17 production by $IL\text{-}1R1^+CD4^+$ T cells stimulated with anti-CD3/CD28 antibodies.[3] The calculations were done using PASS software (Power Analysis and Sample Size, NCSS and PASS). Given the nature of single cell RNA-seq and CyTOF analyses, the sample sizes for these analyses were determined based on our published study which used the same analytic tools.[10] For the experiment of investigating the effect of the anti-IL-1R1 blocking antibody treatment in mice, the sample size calculation was done based on previously published studies in mice and humans.[3,12] The additional details on the sample size calculations were provided in the Supplementary Methods.

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The funding sources for this study had no role in the study design, data collection, analysis, interpretation, or writing of the manuscript.

Results

IL1R1 gene is expressed by S protein-specific memory CD4$^+$ T cells induced by COVID-19 mRNA vaccine in humans as determined by scRNA-seq analysis

We evaluated global genomic characteristics of memory CD4$^+$ T cells specific for S protein, Flu and cytomegalovirus (CMV) in the peripheral blood of healthy subjects who received two doses of COVID-19 mRNA vaccines using multiplexed single-cell RNA sequencing (scRNA-seq). Viral antigen-specific and non-specific memory CD4$^+$ T cells were identified and purified based on OX-40 and 4-1BB expression (Fig. 1a).[13,14] The Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction technique demonstrated heterogeneity in the transcriptomic characteristics of individual cell subsets. CD4$^+$ T cells specific for viral antigens, including S protein, were distinguished from OX40$^-$4-1BB$^-$ CD4$^+$ T cells (Fig. 1b). Gene expression patterns were relatively similar in OX40$^+$4-1BB$^+$ CD4$^+$ T cells specific for different viral antigens, including S protein, Flu and CMV lysates, as compared to unstimulated, OX40$^+$4-1BB$^-$, and OX40$^-$4-1BB$^+$ CD4$^+$ T cells (Fig. 1c) whereas the gene expression pattern of CD4$^+$ T cells specific for S protein (OX40$^+$4-1BB$^+$) were distinct from those of unstimulated and S-protein incubated OX40$^-$4-1BB$^-$, OX40$^+$4-1BB$^-$ and OX40$^-$4-1BB$^+$ CD4$^+$ T cells. We evaluated differentially expressed genes (DEGs) by S protein-specific OX40$^+$4-1BB$^+$ CD4$^+$ T cells in comparison to S protein non-specific OX40$^-$4-1BB$^-$ CD4$^+$ T cells. The DEGs between the two groups (OX40$^+$4-1BB$^+$ vs. OX40$^-$4-1BB$^-$ CD4$^+$ T cells) included ones related to T cell activation, co-stimulation, and immune regulation such as *TNFRSF9* (4-1BB or CD137), *TNFRSF4* (OX40), *TNFRSF18* (GITR or glucocorticoid-induced TNFR-related protein), *IL2RA*, *FOXP3*, and *TIGIT* (T cell immunoreceptor with Ig and ITIM domains) (Fig. 1d) (Supplementary Table S3a–c). Of note, S protein-specific OX40$^+$4-1BB$^+$ CD4$^+$ T cells expressed *IL1R1* and *IL1R2*, which encode IL-1R1 and the decoy IL-1R2, respectively (Fig. 1e), suggesting the possible interaction of these cells with IL-1. We furthered clustering analysis on the OX40$^+$4-1BB$^+$ T cell subset, which identified 4 clusters (Supplementary Figure S1). CD4$^+$ T cells expressing the *IL1R1* and *IL1R2* genes also expressed high levels of *FOXP3* and *IL2RA* genes. The expression of such genes likely reflects antigen mediated CD4$^+$ T cells since T cell receptor trigger is known to transiently upregulate FOXP3 and IL-2RA in CD4$^+$ T cells.[15,16]

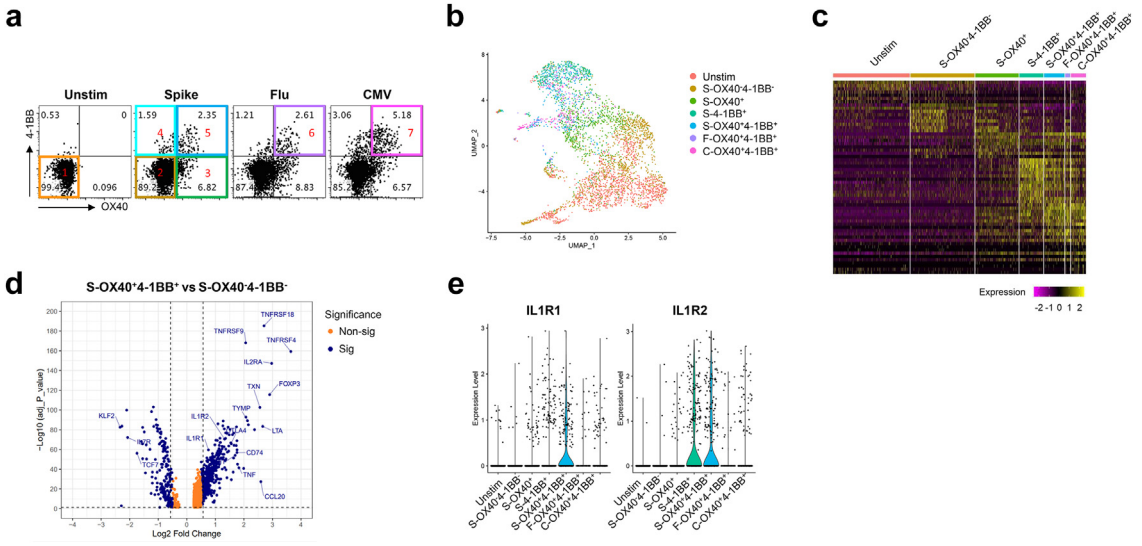

Fig. 1: **Multiplexed single-cell RNA sequencing reveals transciptomic features of SARS-CoV-2 spike (S) protein, influenza virus (Flu) and cytomegalorvirus (CMV)-specific memory CD4$^+$ T cells in healthy subjects**. **(a)** Representative flow cytometry plots of CD4$^+$ T cell subsets (indicated by #1–7) identified based on OX40 and 4-1BB expression following overnight stimulation of PBMCs of COVID-19 mRNA vaccinated healthy individuals with or without S protein overlapping peptides, influenza virus (Flu), or cytomegalovirus (CMV) lysates. scRNA-seq analysis of FACS-purified subsets #1–7 in (A) (n = 2) was done. **(b)** UMAP projection of indicated CD4$^+$ T cell subsets. **(c)** Heatmap showing differentially expressed genes (DEGs) in indicated CD4$^+$ T cell subsets. **(d)** Volcano plot showing DEGs between OX40$^+$4-1BB$^+$ and OX40$^-$4-1BB$^-$ CD4$^+$ T cells in S protein-stimulated PBMCs. **(e)** Plots showing *IL1R1* and *IL1R2* expression in indicated CD4$^+$ T cell subsets.

S protein-specific memory CD4$^+$ T cells induced by COVID-19 mRNA vaccine contain cell subsets (metaclusters) expressing high levels of IL-1R1 and activation-associated molecules at the protein level as determined by CyTOF

In line with the results of scRNA-seq analysis, S protein-specific OX40$^+$4-1BB$^+$ CD4$^+$ T cells expressed high levels of IL-1R1 and IL-1R2 at the protein level as determined by flow cytometry (Fig. 2a, Supplementary Figure S2). We next investigated IL-1R1, IL-1R2, and other molecules by distinct CD4$^+$ T cell populations including OX40$^+$4-1BB$^+$ CD4$^+$ T cells specific for S protein, Flu, and CMV antigens as in Fig. 1a using high-dimensional mass cytometry or Cytometry by Time-Of-Flight (CyTOF). These molecules included ones encoded by DEGs in S protein-specific (OX40$^+$4-1BB$^+$) CD4$^+$ T cells compared to OX40$^-$4-1BB$^-$ CD4$^+$ T cells. As we did previously,[17] we analyzed our CyTOF data by performing PhenoGraph clustering and subsequent metaclustering on CD4$^+$ T cell samples stimulated with or without S protein, Flu and CMV antigens to identify distinct CD4$^+$ T cell subsets based on the expression of the analyzed molecules. PhenoGraph clustering, done independently on each sample, identifies cell subpopulations in each sample. Subsequent metaclustering identifies cell subsets or metaclusters across samples through a secondary clustering analysis on merged PhenoGraph clustering-identified subpopulations from each sample (i.e., inter-sample comparisons)[18,19] (Supplementary Figure S3). Based on the expression levels of the analyzed molecules, cells were clustered into five CD4$^+$ T cell metaclusters by performing PhenoGraph clustering and metaclustering (Fig. 2b and c). Metacluster 1 expressed high levels of Ki-67, FOXP3, IL-1R2, CD25, CD69, FAS, TIGIT and GITR with moderate expression of IL-1R1, T-bet, and CXCR5 (Fig. 2b and c). Metacluster 2 expressed high levels of IL-1R1, CD40L, CTLA-4, T-bet, CD44, CD38, CD69, PD-1 and CXCR5, with moderate expression of CD25 and FAS (Fig. 2b and c). High expression levels of the cell proliferation marker Ki-67 and the cell proliferation promoting co-stimulatory molecule GITR[20] in metacluster 1 suggest that cells in this metacluster exhibit a potent proliferative capacity. Although this cell

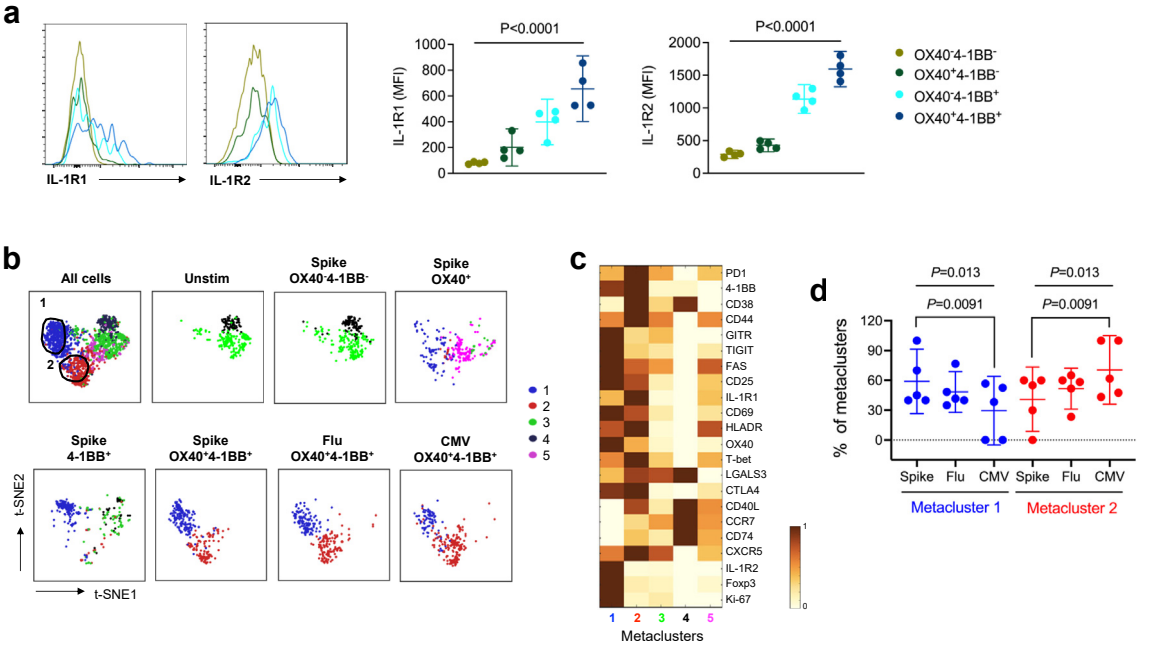

Fig. 2: **SARS-CoV-2 Spike (S) protein-specific memory CD4$^+$ T cells in healthy subjects immunized with COVID-19 mRNA vaccine have a distinct expression pattern of molecules as determined by CyTOF. (a)** Flow cytometric analysis of IL-1R1 and IL-1R2 expression by indicated cell subsets showing expression levels of IL-1R1 and IL-1R2 (mean fluorescence intensity or MFI) in 5 healthy subjects immunized with COVID-19 mRNA vaccine. Left pannels, representative histograms. **(b–d)** CyTOF analysis showing distinct metaclusters in CD4$^+$ T cells specific for S protein, Flu and CMV. PBMCs of COVID-19 mRNA vaccinated healthy individuals were incubated overnight with or without S protein overlapping peptides, Flu or CMV lysates followed by CyTOF analysis. CD4$^+$ T cell populations indicated above the *t*-SNE plots in Fig. 1b were identified in 35 samples from 5 subjects according to the gating strategy as in Fig. 1a and further analyzed using using PhenoGraph and metaclustering. **(b)** *t*-SNE plots showing distinct metaclusters in indicated CD4$^+$ T cells. Numbers 1 and 2 in the *t*-SNE plot of all cells indicate metaclusers 1 and 2, respectively. Unstim, unstimulated. **(c)** Heatmap showing expression levels of indicated molecules by individual metaclusters. **(d)** Frequency of metaclusters 1 and 2 in S protein-, Flu- and CMV-specific OX40$^+$4-1BB$^+$ CD4$^+$ T cells. Bars and error bars indicate mean and 95% CI. *P*-values by one-way ANOVA with Dunnett's post hoc analysis.

subset expressed FOXP3, a marker of regulatory T cells, FOXP3 has also been reported to be expressed transiently in activated and proliferating non-regulatory human CD4$^+$ T cells.[15,16] In contrast, high expression levels of the Th1 cell differentiation transcription factor, T-bet, in metacluster 2 cells support potent effector capacity. Similar cell clusters were found in OX40$^+$4-1BB$^+$ CD4$^+$ T cells specific for Flu and CMV, indicating that viral antigen-specific memory CD4$^+$ T cells can upregulate IL-1R1 and IL-1R2 with T cell activation molecules upon antigenic stimulation (Fig. 2b and c). However, the frequencies of metaclusters 1 and 2 were different in CD4$^+$ T cells specific for S protein, Flu and CMV (Fig. 2d). The CMV-specific CD4$^+$ T cells had an increased frequency of metacluster 2 expressing high levels of IL-1R1 and T-bet compared to S protein-specific CD4$^+$ T cells, which could be related to repetitive stimulation of these CD4$^+$ T cells by latent CMV virus. Metaclusters 3 and 4 expressed low levels of IL-1R1, IL-1R2 and other activation markers such as CD69, CD25, HLA-DR, and CTLA-4 whereas metacluster 5 had moderate levels of IL-1R1, CD25, HLA-DR and CD40L expression (Fig. 2b and c). Indeed, metaclusters 3 and 4 were mostly present in unstimulated and OX40$^-$4-1BB$^-$ CD4$^+$ T cells incubated with S protein which had low levels of *IL1R1* and *IL1R2* gene expression in the scRNA-seq (Fig. 1g), reflecting the unstimulated status of these cells.

IL-1β and anti-IL-1R2 neutralization augmented IFN-γ expression in S protein-specific CD4$^+$ T cells with high levels of IL-1R1 and IL-1R2 expression

IL-1β was reported to promote T cell responses, including cytokine production, although such effect on viral antigen specific T cell responses in humans is largely unknown. A recent study found IL-1β production by human PBMCs incubated with lipid-formulated RNA vaccines,[12] implying the possible role of IL-1β in the development of T cell immune responses to COVID-19 mRNA vaccines. IL-1R1 provides stimulatory signaling while IL-1R2, which has no intracellular signaling domain, acts as a decoy receptor by competing with IL-1R1 for ligands.[21] To determine if IL-1R1 and IL-1R2 expression on S protein-specific CD4$^+$ T cells had biological function, we incubated PBMCs with IL-1β, which substantially increased the frequency of IFN-γ expressing CD4$^+$ T cells at 5 ng/ml in the presence of S peptides (Fig. 3a and b, Supplementary Figure S4). A similar trend was observed with IL-1β at 10 ng/ml although it did not reach statistical significance ($P = 0.058$). This suggests that IL-1β promotes Th1 cell characteristics in S protein-specific CD4$^+$ T cells expressing IL-1R1, although such *in vitro* treatment did not induce IL-17 or IL-21 expression by these cells (data not shown). Of note, IL-1β alone also increased the frequency of IFN-γ$^+$ CD4$^+$ T cells, which was lower than that of the same cells incubated in the presence of both S peptides and IL-1β. To explore the effect of blocking decoy IL-1R2, anti-IL-1R2 neutralizing antibody was added to T cells in the presence of S peptides and IL-1β. The addition of this antibody resulted in an increase in S protein-specific IFN-γ expressing CD4$^+$ T cells (Fig. 3c and d). These findings support the biological implications of IL-1R1 and IL-1R2 expression by human CD4$^+$ T cells specific for viral antigens such SARS-CoV-2 S protein.

COVID-19 mRNA vaccination increased S protein-specific memory CD4$^+$ T cells expressing IL-1R1 in healthy human subjects, correlating with S protein-specific antibody production

We next evaluated the possible effect of repetitive viral antigen stimulation on IL-1R1 and IL-1R2 expressing antigen specific CD4$^+$ T cells *in vivo* by analyzing such cells in healthy subjects following the 1st and 2nd doses of COVID-19 mRNA vaccines using CyTOF (Fig. 4a). Phenograph clustering and metaclustering on pre-gated OX40$^+$4-1BB$^-$, OX40$^-$4-1BB$^+$, OX40$^+$4-1BB$^+$ CD4$^+$ T cells identified 4 metaclusters (Fig. 4b). In S protein-specific OX40$^+$4-1BB$^+$ CD4$^+$ T cells, metaclusters 2 and 4 with distinct characteristics, including IL-1R1 and R2 expression, were identified at both time points (T) 1 and 2 (Fig. 4c). Metacluster 2 expressed high levels of IL-1R1, T-bet, CD40L, CD25, CD69, and PD-1 while metacluster 4 expressed high levels of IL-1R2, Ki-67, FOXP3, CD25, TIGIT and GITR. Metacluster 4 also expressed moderate levels of IL-1R1 although there was low expression of IL-1R2 in metacluster 2. We noticed that metacluster 1, which was present primarily in OX40$^+$4-1BB$^-$, expressed both IL-1R1 and CXCR5 while the expression levels of other activation markers such as HLA-DR, CD25, CD69 and PD-1 were low in this cluster. These findings suggest that some circulating CD4$^+$ T cells such as OX40$^+$4-1BB$^-$ cells which are not activated specifically by antigen may express both IL-1R1 and CXCR5. Following the 2nd dose of COVID-19 mRNA vaccine, the frequency of metacluster 2 increased while the frequency of metacluster 4 decreased (Fig. 4d). Of note, the molecular features of metacluster 2, which increased following the 2nd dose, were similar to those observed in the increased population of CMV-specific CD4$^+$ T cells (Fig. 2b and c). This finding is likely related to repetitive antigen stimulation, which is in line with effector T cell differentiation.[22] We examined the possible relationship of IL-1R1 and R2 expression by S-protein specific CD4$^+$ T cells with S protein-specific humoral immune responses. The expression levels of IL-1R1 on S protein-specific CD4$^+$ T cells measured after the 1st and 2nd doses of COVID-19 mRNA vaccine correlated with serum levels of S protein-specific IgG antibody (Fig. 4e). In contrast, the expression levels of IL-1R2 on the same CD4$^+$ T cells measured after the 1st dose of COVID-19 mRNA vaccine inversely correlated with serum levels of S

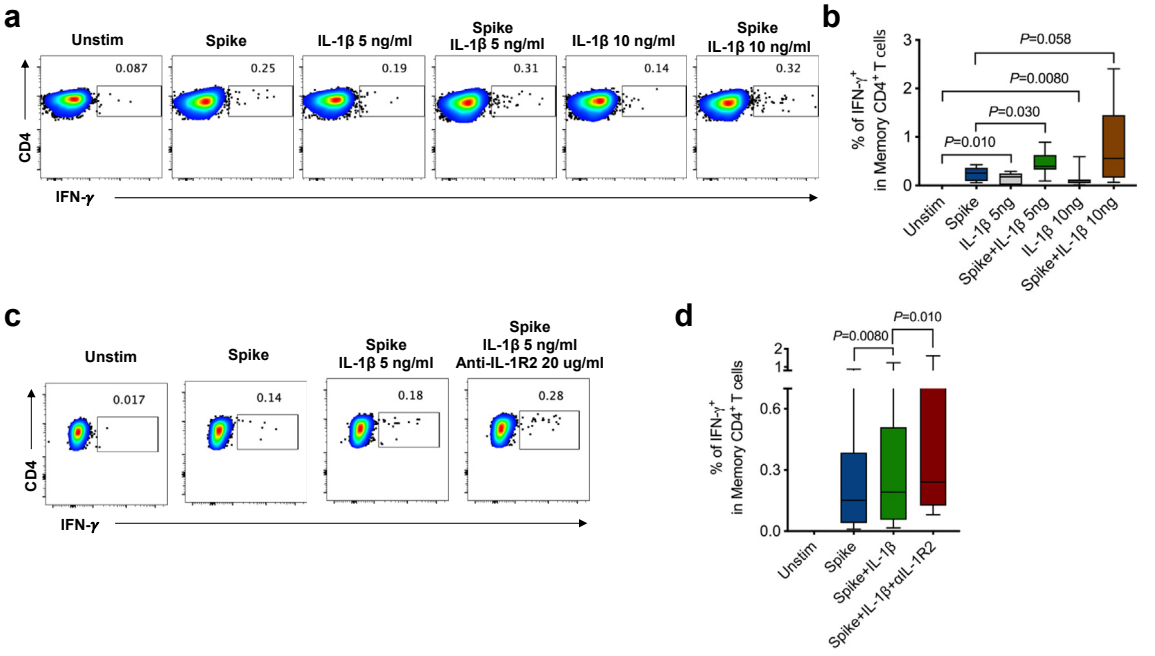

***Fig. 3:* IL-1β with or without anti-IL-1R2 neutralizing antibody enhances IFN-γ$^+$ expression by human CD4$^+$ T cells in response to SARS-CoV2 spike (S) protein. (a–d)** Flow cytometric analysis of IFN-γ$^+$ CD4$^+$ T cells in PBMCs (n = 7 COVID-19 mRNA vaccinated healthy subjects) which were incubated for 3 days in the presence or absence of S protein overlapping peptides (Spike) with or without recombinant human IL-1β (5 or 10 ng/ml) (a–d) or a combination of human IL-1β and anti-IL-1R2 neutralizing antibody (20 μg/ml, c-d). Representive dot plots (a, c). Each dot in scatter graphs (b, d) represents data from indicated conditions. Bars and error bars (Box-and-whisker) indicate median ± interquartile range (b) and (d). *P*-values by the Wilcoxon matched-pairs signed rank test (b) or the Sign test (b, d).

protein-specific IgG (Fig. 4f). Overall, repetitive immune stimulation such as COVID-19 mRNA vaccination likely increases IL-1R1 expression by antigen-specific CD4$^+$ T cells with the possible implication in generating humoral immunity in healthy subjects.

IL-1R1 blocking reduces IFN-γ expression in S protein-specific CD4$^+$ T cells from mice received COVID-19 mRNA vaccine

To further address the systemic role of IL-1R1 in CD4$^+$ T cell responses following mRNA vaccination, we blocked IL-1R1 by injecting anti-IL-1R1 neutralizing antibodies intraperitoneally in mice on 1 day before, on the day, and 1 day after each dose of COVID-19 mRNA vaccine (Fig. 5a). The mice injected with anti-IL-1R1 antibodies had a decreased frequency of S protein-specific CD4$^+$ T cells expressing IFN-γ and TNF-α compared to mice injected with vehicle control (Fig. 5b and c). A similar trend was noticed in S-protein specific CD4$^+$ T cells expressing IL-2, although statistical significance was not reached. The frequency of OX40$^+$4-1BB$^+$ memory CD4$^+$ T cells was decreased in the vaccinated mice treated with the IL-1R1 neutralizing antibodies (Supplementary Figure S5). IL-1R1 neutralization also led to a reduction in serum S protein-specific IgG levels (Fig. 5d), splenic T follicular helper (Tfh) CD4$^+$ T cells, and germinal center B cells (Supplementary Figure S6), which is in line with the results of a previous study reporting the implication of IL-1β in promoting Tfh cell differentiation in mice immunized with ovalbumin and alum.[23] Our findings from both human *ex vivo* and murine *in vivo* studies support the biological implication of IL-1 and its receptor system in modulating CD4$^+$ T cell responses to COVID-19 vaccine.

Patients with primary antibody deficiencies (PADs) show IL-1R1 expression on spike protein-specific CD4$^+$ T cells

IL-1R1 and Toll-like receptors (TLRs), which are essential for the recognition of microbes, share signaling pathway through MyD88 (myeloid differentiation molecule) that recruits IL-1 receptor-associated kinase 4 (IRAK-4).[24] Indeed, human IRAK-4 deficiency is a type of PAD associated with recurrent bacterial infections.[24] Patients with PADs may not be able to develop an effective antibody response to microbes and/or vaccines as a result of B and/or T cell defects.[25] We explored whether patients with PADs could upregulate IL-1R1 on antigen-specific CD4$^+$ T cells by evaluating S protein-specific CD4$^+$ T cells in the setting of COVID-19 mRNA vaccination. Here we divided patients with PADs into two groups with CVID and other PADs, respectively, as we did previously[9] since these conditions

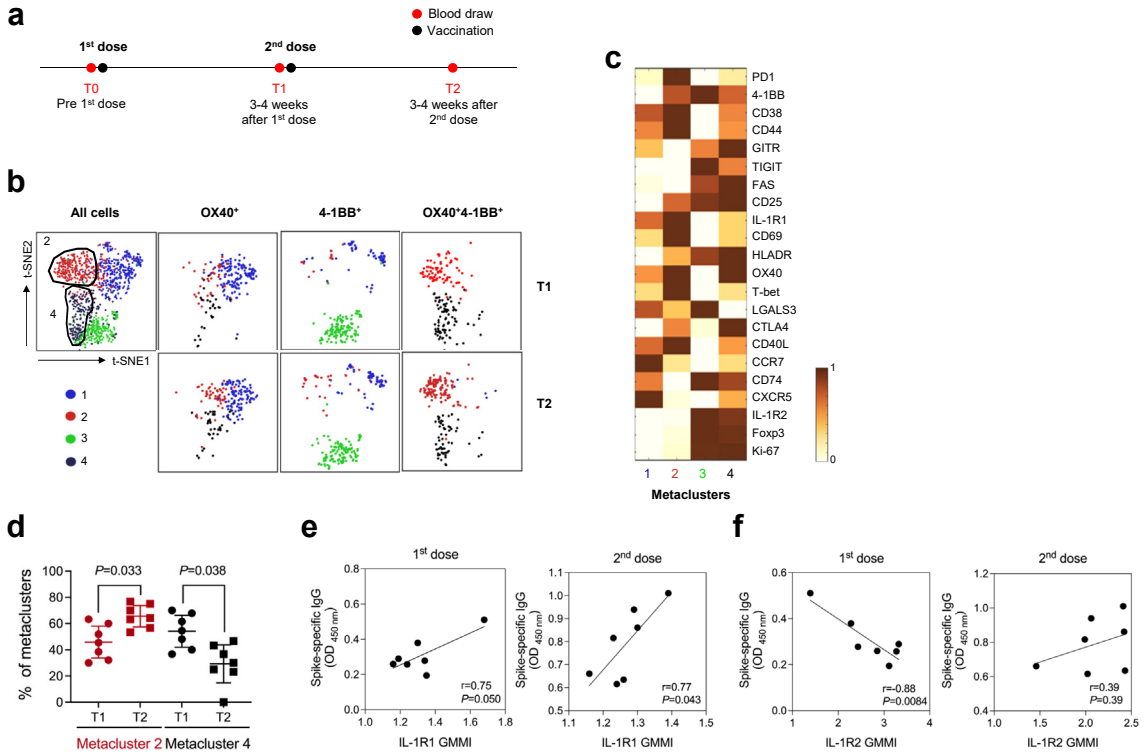

Fig. 4: **IL-1R1 expessing memory CD4⁺ T cells specific for SARS-CoV-2 spike (S) protein increase in healthy subjects following the 2nd dose of COVID-19 mRNA vaccine, correlating with anti-S protein IgG production.** **(a–f)** PBMCs of healthy subjects (n = 7) were obtained before and 3–4 weeks after the 1st and 2nd doses of Pfizer-BioNTech or Moderna COVID-19 mRNA vaccine. Cells were incubated overnight with or without S protein overlapping peptides and analyzed by CyTOF. CD4⁺ T cell populations indicated above the *t*-SNE plots in (b) were identified according to the gating strategy as in Fig. 1a and further analyzed using using PhenoGraph and metaclustering. **(a)** Blood collection time points (T). **(b)** *t*-SNE plots showing distinct metaclusters in indicated CD4⁺ T cell populations at T1 and T2. Numbers 1 and 2 in the *t*-SNE plot of all cells indicate metaclusers 2 and 4, respectively. **(c)** Heatmap showing expression levels of indicated molecules by individual metaclusters. **(d)** Graph showing the frequency of metaclusters 2 and 4 at T1 and T2. Bars and error bars indicate mean and 95% CI. *P*-values were obtained by the paired *t*-test. **(e–f)** The relationship of geometric mean metal intensities (GMMI) of IL-1R1 (e) and IL-1R2 (f) expressed on spike protein-specific OX40⁺4-1BB⁺ CD4⁺ T cells with serum anti-s protein IgG levels. 95% CI for correlation coefficient are 0.0015–0.96 (e, 1st dose), 0.039–0.96 (e, 2nd dose), −0.98 to −0.39 (f, 1st dose), −0.52 to 0.88 (f, 2nd dose). Sera were obtained at 10–14 days after the 1st and 2nd doses of COVID-19 mRNA vaccine. *P* and *r* values were obtained by Pearson correlation.

can have distinct clinical and immunological characteristics.[26] S-protein specific memory CD4⁺ T cells developed by COVID19 mRNA vaccination expressed IL-1R1 in these subjects, although such response appeared to be less in patients with CVID following the 1st dose of the vaccine (Fig. 6a and b). The levels of IL-1R1 did not correlate with the levels of S protein-specific IgG antibody (Fig. 6b). As in healthy subjects, T cell activation associated molecules such as CD40L, CD25, CD69 and CTLA-4 as well as IL-1R2 were expressed with IL-1R1 by S protein-specific CD4⁺ T cells (Fig. 6c and Supplementary Figure S7).

Discussion

T cells can have diverse responses to cytokines, having varying levels of cytokine receptors.[3,27,28] We previously showed two subsets of human naïve and memory CD4⁺ T cells with or without expressing IL-1R1.[3] IL-1β enhanced IL-17 production from naïve and memory IL-1R1⁺ CD4⁺ T cells in the presence of T cell receptor triggering with anti-CD3/CD28 antibodies or IL-23.[3,29] The possible role of IL-1R1 in regulating T cell response was also reported in a mouse study where IL-1R1 on CD4⁺ T cells was shown to be essential for the expression of effector cytokines including IFN-γ and IL-17 in response to ovalbumin and *C. rodentium*.[6] However, it is unknown about the IL-1R1 expression by human CD4⁺ T cells that are activated by pathogens, especially viral antigens in the context of repetitive immune stimulation, and the biological implication of such receptor interface with IL-1 in determining effector function of CD4⁺ T cells. The current study has addressed these questions, which are clinically important

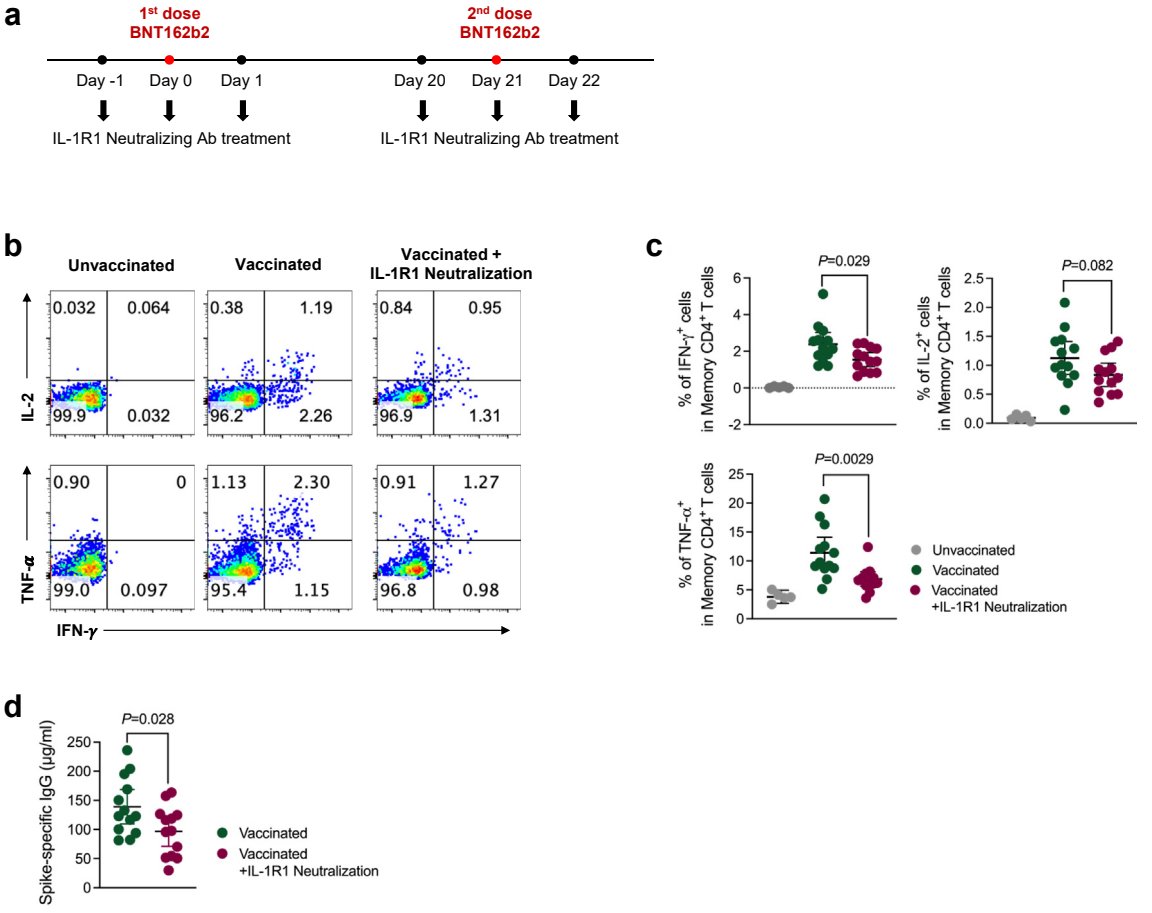

Fig. 5: **Administrating anti-IL-1R1 neutralizing antibody decreases spike protein (S) -specific $CD4^+$ T cells expressing IFN-γ in mice immunized with COVID-19 mRNA vaccine. (a)** Schematic diagrm showing IL-1R1 neutralizing antibody administration schedule in C57BL/6 mice immunized with BNT162b2 COVID-19 mRNA vaccine. **(b–c)** Flow cytometric analysis of IFN-γ^+, TNF-α^+, and IL-2^+ $CD4^+$ T cells in splenocytes from unvaccinated, vaccinated, and vaccinated mice treated with anti-IL-1R1 neutralizing antibody (Ab) (n = 13). For intracelluar cytokine analysis, splenocytes were incubated overnight with or without S protein overlapping peptides. **(d)** Anti-S protein IgG levels were determined in sera from the same mice (n = 13). Bars and error bars indicate mean and 95% CI. *P*-values were obtained by the unpaired *t*-test.

given the role of $CD4^+$ T cells in host defense, by characterizing S protein-specific $CD4^+$ T cells in human subjects in the setting of COVID-19 mRNA vaccination as well as by interrupting the interface of IL-1 and IL-1R1 in animals which received the same vaccine. We found that S protein-specific $CD4^+$ T cells expressed high levels of IL-1R1 in both healthy subjects and patients with PADs. Triggering or blocking of this receptor through IL-1β or neutralizing antibody significantly altered the S protein specific $CD4^+$ T cell responses as determined by cytokine production in human *ex vivo* and murine *in vivo* studies. Overall, these findings support the implication of IL-1R1 and its interface with IL-1 in shaping $CD4^+$ T cell responses to viral antigens such as S protein.

We identified and purified viral antigen-specific $CD4^+$ T cells based on OX40 and 4-1BB expression. These molecules serve as T cell receptor (TCR) dependent activation induced markers (AIM) which are utilized to identify and quantify T cells specific for viruses, vaccines, and other antigens.[13,14,30,31] Since the AIM assay requires only cell surface staining without cell permeabilization, it is suitable for purification of antigen-specific T cells using fluorescence-activated cell sorting (FACS). We also performed antigen-specific intracellular cytokine staining analysis to assess functionality of S protein-specific $CD4^+$ T cells. In the current study, IFN-γ- but not IL-17-expressing human $CD4^+$ T cells were found in response to S protein alone or a combination of S protein and recombinant IL-1β, indicating that COVID-19 mRNA vaccines dominantly induce Th1 cells which are known to be involved in antiviral immune responses. These findings concur with the role of IL-1β in promoting Th1 cell response,[32] although IL-1β is also known to promote Th17 cell responses with

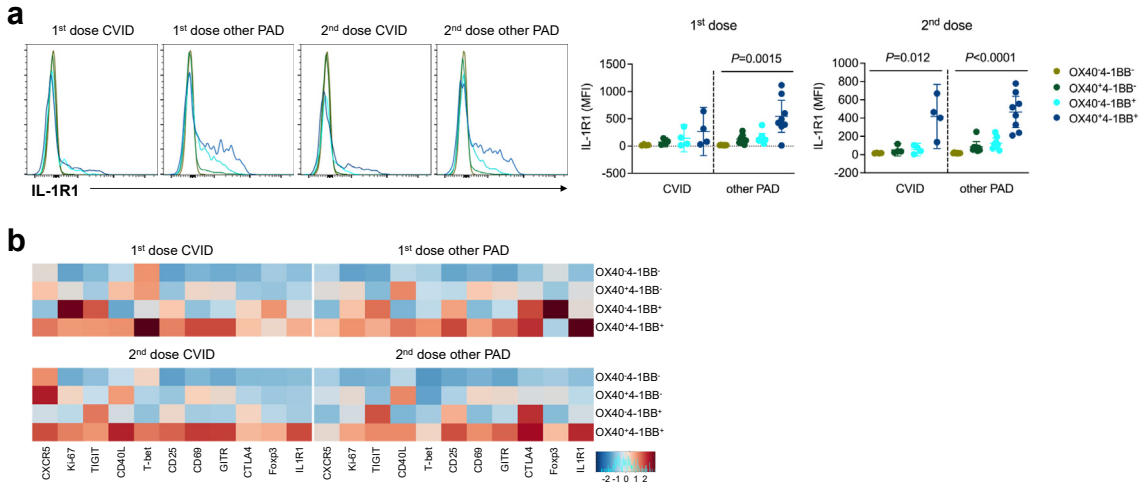

***Fig. 6:* SARS-CoV-2 spike (S) protein-specific memroy CD4$^+$ T cells in patients with primary antibody deficiency (PAD) express IL-1 receptor 1 after COVID-19 vaccination, without showing correlation with serum anti-S protein IgG levels**. PBMCs were obtained from patients with PADs (4 CVID and 8 other PADs) following the 1st and 2nd doses of COVID-19 mRNA vaccine. Cells were incubated overnight with or without S protein overlapping peptides followed by flow cytometric analysis. **(a)** Flow cytometric analysis of IL-1R1 expression by indicated CD4$^+$ T cell subsets. Representative histograms and scatter graphs showing mean fluorescence intensity (MFI) of IL-1R1. **(b)** Heatmap illustrating the expression levels of indicated molecules (z-scores of MFI) on CD4$^+$ T cell subsets defined by the expression of OX40 and 4-1BB. Bars and error bars indicate mean and 95% CI (a). *P*-values were obtained by ANOVA (a).

IL-17 production.[3] We investigated high-dimensional characteristics of memory CD4$^+$ T cells specific for viral antigens including S protein, Flu, and CMV using CyTOF. The high-dimensional characteristics of such cells were similar as determined by PhenoGraph clustering based on expression levels of multiple molecules including those related to cell activation (*e.g.*, CD25, CD69, HLA-DR, CTLA-4, PD-1), proliferation (Ki-67), cytokine regulation (i.e., the Th1 transcription factor T-bet), and cytokine recognition (IL-1R1), suggesting the presence of a shared activation pathway(s) among CD4$^+$ T cells which target different viruses.

We prospectively evaluated the high-dimensional characteristics of S protein-specific CD4$^+$ T cells in healthy subjects before and after receiving the 1st and 2nd doses of COVID-19 mRNA vaccine using CyTOF. Following the 2nd dose of COVID-19 mRNA vaccine, the frequency of S protein-specific CD4$^+$ T cells expressing IL-1R1 and T-bet increased in healthy subjects, indicating that repetitive antigenic stimulation with COVID-19 vaccinations may drive CD4$^+$ T cells towards a Th1 dominant state. This notion is supported by our finding of the latent CMV-specific CD4$^+$ T cells with the expression of IL-1R1 and T-bet (Fig. 2c) as well as by the upregulation of IL-1R1 on human memory CD4$^+$ T cells by TCR triggering with anti-CD3/CD28 antibodies.[5] The high-dimensional characteristics of S protein-specific CD4$^+$ T cells in our CyTOF analysis is in line with the results of our examination of a publicly available scRNA-seq dataset on human CD4$^+$ T cells from healthy subjects immunized with COVID-19 mRNA vaccine (Supplementary Figure S8). This analysis revealed a CD4$^+$ T cell cluster expressing genes encoding IL-1R1, IL-1R2 and other activation molecules such as CD25 and CTLA-4. This cluster increased after COVID-19 mRNA vaccination, implying that the vaccination effect on such molecules in CD4$^+$ T cells occur at both gene and protein levels. CD4$^+$ T cells are known to promote humoral immunity by providing help to B cells for activation and immunoglobulin switching via secreting cytokines and expressing stimulatory molecules such as CD40L. The S protein-specific IL-1R1$^+$ CD4$^+$ T cell cluster which increased after the 2nd dose of COVID-19 mRNA vaccine (i.e., Cluster T2 in Fig. 4b–d) expressed high levels of CD40L. In fact, the expression levels of IL-1R1 on S protein-specific CD4$^+$ T cells appeared to correlate with serum anti-S protein IgG levels in healthy subjects. These findings support that the implication of IL-1R1 expression by viral antigen specific CD4$^+$ T cells could extend beyond the enhancement of T cell effector cytokines, potentially affecting humoral immunity as well.

Patients with PADs, including common variable immune deficiency (CVID), IgG subclass 2 deficiency (IgG2D), IgG deficiency (IgGD), and specific antibody deficiency (sAbD), can have impaired antibody response to pathogens and/or vaccines.[25,33] We previously reported that patients with IgGD, IgG2D and sAbD mounted anti-S IgG responses at the levels comparable to healthy controls following the 1st and 2nd doses of the COVID-19 mRNA vaccines although some patients with CVID could develop decreased anti-S IgG

responses after the 2nd dose of the vaccines.[9] Also, we found that both CVID and other PAD patients had an increased frequency of S protein-spike $CD4^+$ T cells following the vaccination as determined by OX40 and 4-1BB expression.[9] After the 2nd dose of COVID-19 vaccine, the cellular characteristics of such cells, including expression of IL-1R1, CD40L, CD25 and CD69, in patients with CVID and other PADs appeared to be largely similar to those in healthy subjects, suggesting that the functional quality of S protein-specific $CD4^+$ T cells may remain intact in these patients. However, we did not find a significant relationship of IL-1R1 expression by S protein-specific $CD4^+$ T cells with anti-S protein antibody levels in patients with PAD. These findings are in line with the results of a recent study showing robust $CD4^+$ T cell responses to spike protein in patients with CVID who were followed up to 22 months after the first dose of COVID-19 immunization although they had inadequate humoral immune responses to the vaccines.[34]

The cytokine IL-1β is produced primarily by innate immune cells including monocytes. A recent study showed IL-1β production by human PBMCs stimulated with lipid formulated RNA vaccines through activating Toll-like receptors (TLRs) and the NLRP3 inflammamsome,[12] alluding to the role of IL-1β in generating immune responses to COVID-19 mRNA vaccines. Indeed, in human PBMCs stimulated with such RNA vaccines, $CD14^+$ monocytes were the primary source of IL-1β which was an upstream inducer of multiple inflammatory cytokine production from the same stimulated PBMCs.[12] Hyperinflammation following mRNA and DNA COVID-19 vaccinations has been successfully treated with IL-1 blocking anakinra, a recombinant IL-1R antagonist.[35] However, there is no study reporting suppression of COVID-19 vaccine responses in patients undergoing IL-1 blockade therapy. COVID-19 mRNA vaccines could have a dual effect by inducing IL-1β production from myeloid cells, including monocytes and macrophages, and modulating its receptor system on $CD4^+$ T cells, especially in the setting of repetitive vaccinations. COVID-19 mRNA vaccine-induced IL-1β can affect S protein-specific $CD4^+$ T cells which express IL-1R1, leading to enhanced S protein-specific $CD4^+$ T cell responses. Indeed, this notion is supported by our findings of an increase in the median frequency of IFN-γ^+ $CD4^+$ T cells incubated with S protein in the presence of IL-1β based on an analytic approach proposed by published studies.[36,37] We speculate that such effect could offer advantages to individuals receiving multiple doses of COVID-19 vaccines, even those designed for different variants, by maintaining, expanding, and/or activating pre-existing S protein-specific memory $CD4^+$ T cells which can provide protection against diverse SARS-CoV-2 variants through the mechanism of cross-reactive immunity.[38] Of note, a body of evidence supports that inflammasome activation and IL-1β production occur in COVID-19, positively correlating with adverse clinical outcomes.[39] The IL-1R antagonist anakinra was approved for severe COVID-19 by the European Medicines Agency[39] while the United States Food and Drug Administration had issued an Emergency Use Authorization (EUA) for the emergency use of anakinra for hospitalized adults with COVID-19 pneumonia requiring supplemental oxygen who were at risk of progressing to severe respiratory failure and likely to have an elevated plasma soluble urokinase plasminogen activator receptor.[40] Although this suggests the pathogenetic role of IL-1β in COVID-19, our findings imply that IL-1β and IL-1R1 could serve as molecules that mediate an interaction between myeloid cells, including monocytes and macrophages, and $CD4^+$ Th cells for the development of adaptive immune responses to SARS-CoV-2. We recognize the limitations of our study which include relatively small sample sizes, assessment of normality assumption in small sample sizes, and wide confidence intervals. Also, our study was not powered to evaluate how our biological findings could be associated with the efficacy of COVID-19 vaccines.

To the best of our knowledge, our study is the original one to demonstrate the expression of IL-1R1 by viral antigen-specific human $CD4^+$ T cells and the functional implication of such receptor expression, especially in the context of repetitive antigenic stimulation with vaccination. Also, our high-dimensional single cell CyTOF analysis indicates that repetitive immunization with COVID-19 mRNA vaccines can expand S protein-specific $CD4^+$ T cells expressing IL-1R1, shaping the viral antigenic $CD4^+$ T cells to acquire enhanced capacity to interact with innate immune molecules such as IL-1. The latter point is supported by the results of a recent study showing IL-1β production from human peripheral blood mononuclear cells (PBMCs) incubated with lipid-formulated RNA vaccines,[12] which implies the role of IL-1β in the development of T cell immune responses to COVID-19 mRNA vaccines. Our study noticeably advances this finding by demonstrating IL-1R1 expression on S protein-specific $CD4^+$ T cells and its functional implication in the development of such responses. Taken together, the results of our study offer crucial insights into the fields of anti-viral immunity and vaccine development by highlighting the role of the interface of IL-1 and its receptor system in generating potent cellular immune responses to viral antigens.

Contributors

HJP and MSS designed the study, performed the experiments, analyzed and interpreted the results, and participated in writing the manuscript; JJS, JP-Y, SU, YA and JY recruited the subjects, performed the experiments, and participated in writing the manuscript; HSK, BK, MHK, SJL, SJ, SYY, and MKR analyzed and interpreted the results, and participated in writing the manuscript; RB and IK designed the study, analyzed and interpreted the results, participated in writing the manuscript and supervised the research. HJP and IK have verified the underlying data. All authors read and approved the final version of the manuscript.

Data sharing statement

All data are available in the main text or the supplementary materials. Single-cell RNA-seq raw data and processed files are publicly available in the Gene Expression Omnibus under accession code GSE206134.

Declaration of interests

MKR is an employee of Quest Diagnostics. IK and RB received research funding from Quest Diagnostics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105114.

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