Cross-reactive T cells enhance immune responses in SARS-CoV-2 infection and vaccination

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The functional relevance of pre-existing cross-immunity to SARS-CoV-2 is a subject of intense debate. Utilizing JPT’s PepMix™ peptide pools, we show that human endemic coronavirus (HCoV)-reactive $CD4^+$ T cells with some being SARS-CoV-2-cross-reactive are ubiquitous but decrease with age. Detailed analysis of T cell epitopes, using the SARS-CoV-2 spike glycoprotein epitope mapping peptide set (EMPS), identified a universal immunodominant human coronavirus-specific spike peptide ($S_{816-830}$). Pre-existing spike and $S_{816-830}$-reactive T cells were recruited into immune responses to SARS-CoV-2 infection and their frequency correlated with anti-SARS-CoV-2-Subunit-1-IgG titers. Spike protein cross-reactive T cells were also activated after primary BNT162b2 (Comirnaty®) COVID-19 mRNA vaccination displaying kinetics similar to secondary immune responses. Moreover, using Peptide ELISA, we demonstrate that the rapid T cell response is accompanied by early production of antibodies targeting a cross-reactive epitope in the spike glycoprotein. Our results highlight the functional contribution of pre-existing spike-cross-reactive T cells in SARS-CoV-2 infection and vaccination. Cross-reactive immunity may account for the unexpectedly rapid induction of immunity following primary SARS-CoV-2 immunization and the high rate of asymptomatic/mild COVID-19 disease courses.

Introduction

The majority of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected individuals experience an asymptomatic or mild course of coronavirus disease 2019 (COVID-19). However, severe or fatal disease occurs in about 5% of those infected and is primarily associated with advanced age and comorbidities such as diabetes, chronic cardiovascular, pulmonary, and kidney diseases[1]. Given that SARS-CoV-2 is a newly emerged human pathogen, it was initially assumed that SARS-CoV-2 encounters an immunologically naive population. However, SARS-CoV-2 displays considerable homologies with endemic, ‘common cold’ human coronaviruses (collectively referred to as "HCoV")[2,3]. There is now strong evidence for cellular and humoral cross-reactivity to SARS-CoV-2[3–14], but whether HCoV mediated cross-reactive immunity is beneficial in SARS-CoV-2 infection has remained unclear[2,8,15,16]. While recent HCoV infection is associated with less severe COVID-19, suggesting a protective role[17], other studies did not find such correlation. A better understanding of the extent and impact of cross-immunity in SARS-CoV-2 infection and vaccination is needed, as cognate cross-immunity might not only influence disease course but also the efficacy of vaccination regimens.

Results

A striking feature of SARS-CoV-2 infection is the strong positive correlation of age with disease severity. Immunosenescence, which offers a probable explanation for this, is associated with a lack of newly generated T cells. Instead, small numbers of clones resulting from persistent infections are expanded which limits the breadth and quality of T cell responsiveness[18,19].

Previously we demonstrated that cross-reactivity to a PepMix™ covering S_{1-640}, i.e. the N-terminal S-I spike glycoprotein peptide subpool, was rare in unexposed individuals, whereas cross-reactivity could be detected more frequently with the corresponding C-terminal S-II peptide subpool (covering $S_{636-1273}$) which displays higher homology to the HCoVs[3]. To assess the impact of age on SARS-CoV-2-(cross)-reactive T cell immunity, we examined SARS-CoV-2 S-II spike-specific $CD4^+$ T cell responses in 568 unexposed individuals and 174 COVID-19 convalescents (Fig. 1). COVID-19 convalescents displayed an age-associated increase in spike S-II-reactive T cells in agreement with higher disease severity in the elderly. Cross-reactivity in unexposed individuals, by contrast, was found in more individuals and was of greater magnitude among younger people but less frequent and of lower magnitude among older people. T cells reacting to a control peptide pool representing a mixture of selected T cell epitopes from common pathogens (CEFX pool) remained relatively stable with age.

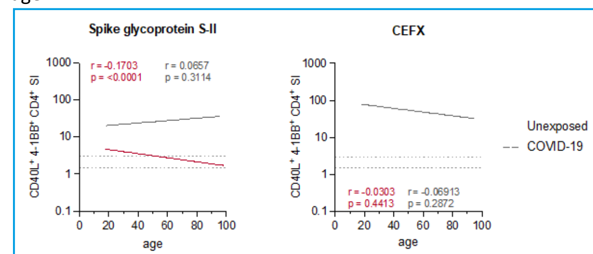

Fig.1: Magnitude of SARS-CoV-2-T-cell-cross-reactivity decreases with age. Scatter plots show the stimulation index (SI) ($CD40L^+41BB^+$ $CD4^+$ T cells) among PBMCs stimulated with SARS-CoV-2 S-II (peptide pool covering the C-terminal part of spike glycoprotein), or CEFX (known T cell-stimulating peptides from CMV, EBV, flu and other common pathogens) plotted against age in *n*=568 unexposed donors and *n*=174 COVID-19 convalescents. Dotted lines indicate an SI of 1.5 and 3.

We next sought to identify the cross-reactive epitopes within the SARS-CoV-2 spike glycoprotein. For that purpose, short-term $CD40L^+4\text{-}1BB^+$ SARS-CoV-2 spike glycoprotein reactive $CD4^+$ T cell

lines were restimulated with the Epitope Mapping Peptide Set SARS-CoV-2 (JPT). We identified and validated two overlapping T cell stimulating peptides – referred to as peptide 204 (N´-SKRSFIEDLLFNKVT-C´, aa 813-827) and peptide 205 (N´-FIEDLLFNKVTLADA-C´, aa 817-831) in five of five donors analyzed (Fig. 2). Two donors also responded to additional peptides (peptides 188, 189, 240, and 251; Fig. 2). Sequence alignment revealed that, together, peptides 204 and 205 covered the fusion peptide domain of spike, which is characterized by strong homology with other HCoVs. By analyzing additional 15-aa peptides along the sequence covered by the peptides 204 and 205, we identified the sequence N´-SFIEDLLFNKVTLAD-C´ (aa 816-830) as an immunodominant pan-coronavirus T cell reactive peptide (data not shown).

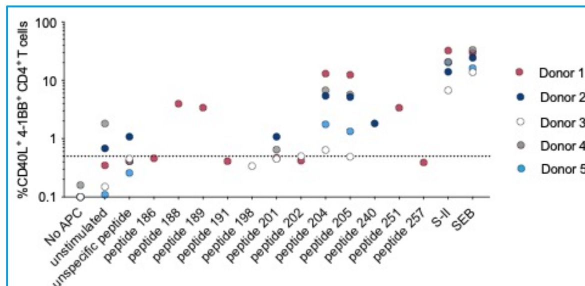

Fig.2: Identification of the immunodominant cross-reactive peptide sequence. Expanded SARS-CoV-2 S-II reactive CD40L^{+}4-1BB^{+} CD4^{+} T cells were restimulated with single peptide candidates determined from matrix stimulation in the presence of autologous feeder cells. Readout was the frequency CD40L^{+}4-1BB^{+} reactive CD4^{+} T cells. The cut-off was set at a factor of 0.5 relative to unstimulated controls.

One important open question was whether SARS-CoV-2-cross-reactive T cells influenced the disease course of primary SARS-CoV-2 infection, and if so, to what extent. Whilst monitoring healthy unexposed study participants for primary SARS-CoV-2 infection we identified 17 cases of acute primary SARS-CoV-2 infection. All 17 cases showed detectable virus titers and a mild to moderate COVID-19 disease course (no hospitalization required). Robust CD4^{+} T cell responses specific of SARS-CoV-2 spike subpools S-I and S-II as well as Subunit 1 (S1)-specific IgG antibody responses were detected (data not shown). Anti-SARS-CoV-2-S1 binding antibody (IgG) units (BAU) and neutralizing antibody titers after infection clearance (day 29-71 after infection) positively correlated with S-II-cross-reactive T cell levels measured at d0 (prior to infection) suggesting that pre-existing cross-reactive CD4^{+} T cells enhance SARS-CoV-2-specific humoral immunity (Fig. 3).

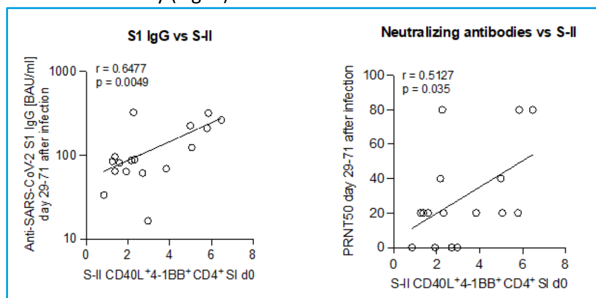

Fig. 3: Presence of HCoV-specific SARS-CoV-2-cross-reactive T cells correlate with higher antibody titers after infection clearance. Scatter plots show the relationship between anti-SARS-CoV-2 S1 IgG antibody levels (OD, left graph) and neutralizing antibody titers (PRNT50, right graph) after infection clearance (day 29-71) and the SI of CD40L^{+}4-1BB^{+} CD4^{+} T cells upon S-II stimulation prior to infection (d0). n = 17.

Finally, we investigated how pre-existing SARS-CoV-2 S-II-cross-reactive T cells in healthy unexposed individuals might influence the course of BNT162b2 COVID-19 spike mRNA vaccine responses. We monitored baseline- and follow-up humoral- and T cell-responses against SARS-CoV-2- and HCoV spike glycoproteins in 31 healthy adults who underwent primary (day 0) and booster (day 21) vaccination with BNT162b2. Primary vaccination induced robust spike specific CD4^{+} T cell responses in all individuals. These were only slightly enhanced by booster vaccination. High S1-specific IgG antibody titers were detected in all individuals after the 2nd vaccine dose (data not shown). CD4^{+} T-cells from 29 of 31 donors (94%) responded to the HCoV-cross-reactive peptide $S_{816-830}$ at days 7 and 14 (Fig. 4). Of note, kinetics showed a sharp increase from baseline to day 7 but not thereafter, indicative of secondary response kinetics of $S_{816-830}$-reactive T cells. The peptide $S_{809-826}$ (overlapping with $S_{816-830}$) has been identified as dominant target of cross-reactive antibodies in spike glycoprotein peptide screenings[20]. A humoral response to the peptide was detectable as early as 7 days after primary vaccination and was distinct from the slower anti-SARS-CoV-2-S1-IgG response, supporting the notion of secondary response kinetics mediated by pre-existing cross-reactive immunity.

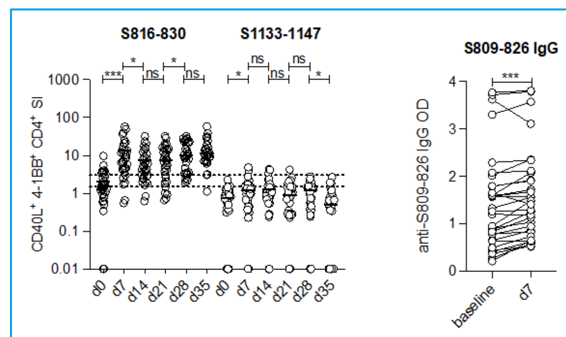

Fig. 4: Peptide S816-830 specific, SARS-CoV-2-cross-reactive T cells are recruited into the BNT162b2 vaccine response accompanied by rapid humoral response. SI of CD40L^{+}4-1BB^{+} CD4^{+} T cells after stimulation with peptide S816-830 and control peptide S1133-1147 at baseline and indicated time points (left graph) and OD of anti-S809-826-peptide IgG ELISA from sera before and 7 days after primary vaccination (right graph). n = 31. *$P<0.05$, **$P<0.01$, ***$P<0.001$ and ns for $P>0.05$ (Student's *t* test).

Materials & Methods

Ex vivo T cell stimulation

Freshly isolated PBMCs were cultivated at a concentration of $5*10^{6}$ PBMC/ml in AB-medium containing RPMI 1640 medium (Gibco) supplemented with 10% heat inactivated AB serum (Pan Biotech), 100 U/ml of penicillin (Biochrom), and 0.1 mg/ml of streptomycin (Biochrom). Stimulations were conducted with PepMix^{TM} overlapping peptide pools (15 aa lengths with 11 aa overlaps, JPT Peptide Technologies) covering the C-terminal part of spike glycoprotein (S-II) or the single peptide $S_{816-830}$ (N´-SKRSFIEDLLFNKVT-C´) or the control peptide $S_{1133-1147}$ (N´-VNNTVYDPLQPELDS-C´) (all JPT Peptide Technologies). All stimulations (peptide pools and single peptides) were performed at a final concentration of 1 µg/ml per peptide. Peptide solvent alone (DMSO), at the same concentration as in peptide-stimulated tubes, was used as a negative control. The CEFX Ultra SuperStim pool (1

µg/ml per peptide) (JPT Peptide Technologies) was used as positive stimulation control. For optimized costimulation, purified anti-CD28 (clone CD28.2, BD Biosciences) was added to each stimulation at a final concentration of 1 µg/ml. Incubation was performed at 37˚C, 5% CO_2 for 16 hours in the presence of 10 µg/ml brefeldin A (Sigma-Aldrich) during the last 14 hours. $CD4^+$ T cell activation was calculated as a stimulation index (SI)=% of $CD40L^+4\text{-}1BB^+$ $CD4^+$ T cells in the stimulation / % of $CD40L^+4\text{-}1BB^+$ $CD4^+$ T cells in the unstimulated control. Dotted lines indicate an SI of 1.5 (probable positive) and 3 (definite positive).

T cell enrichment and expansion

Activated cells were enriched from stimulated PBMCs by magnetic cell sorting (MACS). Cells were stimulated with S-II PepMix™ in the presence of 1 µg/ml of purified anti-CD28 (clone CD28.2, BD Biosciences) and 1 µg/ml of purified anti-CD40 (5C3, Biolegend) for 16 hours followed by staining with anti-CD40L-APC (5C8, Miltenyi) and anti-4-1BB-PE (4B4-1, BD). The activated cells were enriched using anti-PE MultiSort MicroBeads (Miltenyi) according to the manufacturer's instructions. After release of anti-PE beads a second, analogous enrichment step was performed using anti-APC MicroBeads (Miltenyi). The purity of the enriched population was routinely checked to >80% of live cells. Feeder cells were obtained from the 4-1BB-PE negative fraction of the initial enrichment step by CD3 MicroBeads (Miltenyi) depletion and subsequent irradiation at 50Gy. Enriched $CD40L^+4\text{-}1BB^+$ cells were co-cultured with feeder cells at a ratio of 1:1 in AB-medium supplemented with 10 ng/ml of IL-7 and 10 ng/ml of IL-15 (both from Miltenyi) for 10 days followed by 2 days of cytokine starvation. For epitope identification, the restimulation was conducted with the Epitope Mapping Peptide Set SARS-CoV-2 (JPT) according to the manufacturer's instructions in the presence of CD3-depleted, autologous feeder cells.

Flow Cytometry

Stimulations were stopped by incubation in 2 mM EDTA for 5 min. Antibody staining was performed in the presence of 1 mg/ml of Beriglobin (CSL Behring) with fluorochrome-conjugated antibodies titrated to their optimal concentrations. For further details see Loyal et al.[21]. All samples were measured on a MACSQuant® Analyzer 16 (Miltenyi).

Subunit 1 (S1)-IgG ELISA

See Loyal et al.[21].

Peptide ELISA

400 nM of biotinylated peptide $S_{809\text{-}826}$ (Biotin-Ttds-PSKPSKRS*FIEDLLFNKV*-OH, JPT Peptide Technologies) was immobilized on a 96 well Streptavidin plate (Steffens Biotechnische Analysen GmbH) for 1 hour at RT. After blocking serum samples were diluted 1:100 and incubated for 1 hour at 30°C. Subsequently, the plates were washed and HRP-coupled, anti-Human-IgG secondary antibody (Jackson ImmunoResearch) was diluted 1:5000 and added to the wells. After 1-hour incubation at 30°C, HRP substrate was added (TMB, Kem-En-Tec). The reaction was stopped by adding sulfuric acid and absorption was measured at 450 nm using a FlexStation 3.

References

1. Williamson *et al.* Factors associated with COVID-19-related death using OpenSAFELY. *Nature* (2020).
2. Stervbo et al. Epitope similarity cannot explain the pre-formed T cell immunity towards structural SARS-CoV-2 proteins. *Scie Rep* (2020)
3. Braun *et al.* SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* (2020)
4. Grifoni *et al.* Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* (2020)
5. Nelde *et al.* SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. *Nature Immunol* (2021)
6. Le Bert *et al.* SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* (2020)
7. Mateus *et al.* Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science* (2020)
8. Bacher *et al.* Low-Avidity CD4+ T Cell Responses to SARS-CoV-2 in Unexposed Individuals and Humans with Severe COVID-19. *Immunity* (2020)
9. Anderson *et al.* Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. *Cell* (2021)
10. Mishra *et al.* Immunoreactive peptide maps of SARS-CoV-2. *Communications Biology* (2021)
11. Ferretti *et al.* Unbiased Screens Show CD8+ T Cells of COVID-19 Patients Recognize Shared Epitopes in SARS-CoV-2 that Largely Reside outside the Spike Protein. *Immunity* (2020)
12. Ng *et al.* Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science* (2020)
13. Weiskopf *et al.* Phenotype and kinetics of SARS-CoV-2–specific T cells in COVID-19 patients with acute respiratory distress syndrome. *Science Immunology* (2020)
14. Sekine *et al.* Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. *Cell* (2020)
15. Lehmann at al. Deconvoluting the T Cell Response to SARS-CoV-2: Specificity Versus Chance and Cognate Cross-Reactivity. *Front Immunol* (2021)
16. Lee et al. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. *Nature Microbiology* (2020)
17. Sagar *et al.* Recent endemic coronavirus infection is associated with less-severe COVID-19. *J Clin Invest* (2021)
18. Kohler et al. Life after the thymus: CD31+ and CD31- human naive CD4+ T-cell subsets. *Blood* (2009)
19. Farber et al. Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol* (2014)
20. Holenya *et al.* Peptide microarray-based analysis of antibody responses to SARS-CoV-2 identifies unique epitopes with potential for diagnostic test development. *Europ J of Immunol* (2021)
21. Loyal *et al.* Cross-reactive CD4+ T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. *Science* (2021)

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