Progressive and parallel decline of humoral and T cell immunity in convalescent health care workers with asymptomatic or mild-moderate SARS-CoV-2 infection

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Summary

Twenty-two convalescent health care workers had a progressive decrease in specific antibodies against SARS-CoV-2 (41% seroreverted) and T-cell response (75% lost response to spike protein) in a 5.1 months-period, associated with a lower initial adaptative immune response.

Abstract

We investigated the duration of humoral and T-cell immune response in paired samples among 22 convalescent health care workers (HCWs). A median of 1.8 months after diagnosis, T-cell response was significantly lower in HCWs with early loss of antibodies (6 cases, 27%). After 5.1 months, antibodies decline was observed in 77% of cases (41% seroreverted; $p<0.01$), and 36% have lost T-cell response (75% lost response to spike protein). Persistence of immune response was observed in those who developed a greater adaptative immune response. Our data point to the initial immune response as the relevant player in COVID-19 duration of protection.

Keywords: SARS-CoV-2; immune response; duration; convalescent; adaptative immune response; healthcare workers

Background

It is estimated that 40–75% of COVID-19 infections could be mild or asymptomatic [1], and previous studies have demonstrated that humoral immune response wane quickly after infection [2]. Nevertheless, it has been shown that convalescent patients harbored polyfunctional SARS-CoV-2-specific T cells that display a stem like memory phenotype [3] although in a recent study, SARS-CoV-2-specific CD4+ and CD8+ T-cells declined with a half-life of 3-5 months [4].

Of importance, the susceptibility to re-infection could be hypothetically higher in the fraction of the SARS-CoV-2-infected population with particularly low immune memory, especially in the case of close and continued contact with the virus such as frontline health care workers (HCWs). Thus, we focused this study on a cohort of convalescent HCWs who were prospectively assessed after the infection to ascertain the persistence of humoral and CD4+ and CD8+ T-cell response against SARS CoV-2.

Methods

This study was the continuation of a cross-sectional study performed in May 2020 at a tertiary university hospital. At that time, convalescent HCWs underwent blood analysis to evaluate the presence of humoral and reactive T-cell immune response. Then, they were reevaluated after 6 months to ascertain the evolution of humoral and cellular response.

At inclusion, age, sex, body mass index (BMI), presence of comorbidities, smoking, alcohol intake, concomitant medications, COVID-19 symptoms and signs, time of exposure to COVID-19 patients, and exposure to aerosol-generating procedures were collected. Likewise, immunocompromised HCWs (active cancer, HIV infection, transplant, chronic use of corticosteroids or immunosuppressive drugs) were excluded in order to avoid variations in the immune response unrelated to infection.

Mild disease was defined as the presence of symptoms attributable to COVID-19 in absence of radiological infiltrates and lack of hypoxemia (oxygen saturation ≥95% on room air). Moderate disease was defined in case of radiological infiltrates with oxygen saturation ≥95% on room air. The initial cross-sectional study was approved by our ethic committee (EC162/20) and registered at clinicaltrials.gov (NCT04402827). Written informed consent was obtained from all the participants.

Both at inclusion and at the end of follow up, the presence of antibodies was assessed by SARS-CoV-2 ELISA (COVID-19-SARS-CoV-2 IgG ELISA, Demeditech, Germany; positivity threshold 11 relative units (RU)/mL).

The presence of cellular immune response was assessed at the same time points. SARS-CoV-2-specific CD4+ and CD8+ T-cells were measured using *in vitro* stimulation with SARS-CoV-2 peptide pools of viral proteins encompassing the spike (S), membrane (M), and nucleocapsid (N), followed by quantitation of CD4+ and CD8+ T-cell specific interferon (IFN)-γ in live cell flow cytometry, using peripheral blood mononuclear cell (PBMC) samples from all subjects. It was considered significantly reactive if the proportion of positive cells in stimulated wells was at least 2-fold higher in comparison with the negative control wells (unstimulated). A cumulative SARS-CoV-2-specific CD4+ and CD8+T cell measurement was calculated as the sum of the S-, M-, and N-specific CD4+ and CD8+ T cells. A detailed description is included as a supplementary file (see internet-only Supplementary Methods).

In parallel, PBMCs plated in 96-well flat-bottom plates at $7x10^5$ cells/well in complete medium were stimulated for 16 hours with SARS-CoV-2 peptide pools of proteins S, M, and N. Supernatants were harvested for cytokine multiplex quantification in duplicate using a human Th cytokine panel (Human MACSPlex Cytokine 12 kit, Miltenyi-Biotec, Germany). Fifty µL of plasma was mixed with beads coated with capture antibodies specific for IFN-γ, TNF-α, IL12, GM-CSF, IL10, IL4, IL5, IL17A, IL2, and IL6, and analyzed by flow cytometry on a MACSQuant Analyzer using MACSQuantitify software. Results were considered as

significant if there was at least 2-fold increase in cytokine levels in stimulated wells compared to unstimulated wells.

Statistical Analysis

Characteristics of convalescent HCWs were analyzed globally and according to the persistence of immune response. Comparisons between groups were performed using two-tailed statistical tests, chi-square or Fisher's exact tests for categorical variables, and Mann-Whitney test or 1-way analysis of variance (Kruskal-Wallis test) with Dunn's correction for multiple comparisons, as appropriate. Statistical significance was defined as two-sided p values <0.05. Statistics were performed with SPSS, v 23.0.

Results

The COVEX study included 22 HCWs (median age, 34 years; 10 females and 22 males). Detailed characteristics of enrolled individuals are shown in **Supplementary Table 1**. Briefly, 7 HCWs (32%) had asymptomatic infections, 13 referred mild symptoms (59%), and only 2 HCWs had moderate COVID-19. None required hospital admission or received corticosteroid therapy. Nineteen out of 22 participants underwent the RT-PCR SARS-CoV-2 test during the acute phase of infection (13 positive, 6 negative) and 3 were not tested due to laboratory overload and were identified by the presence of suggestive symptoms and seroconversion. All the patients had both rapid IgG qualitative test and specific ELISA IgG positive results after the infection. Significant differences were observed between severity subgroups, with asymptomatic being younger (29 vs 39 years, p=0.005), and with a no significant trend to have negative RT-PCR (43% vs 20%).

Initial evaluation was performed a median time of 55 days (interquartile range, 36-65) after diagnosis. At this time, six individuals (27%) had negative results in our serologic diagnostic tests. Although not significant, individuals who seroreverted were younger (33.3 vs 40.7), and asymptomatic (50% vs 33%), and there were no differences in time since diagnosis (52 vs 58 days). IFN-γ producing CD4+ or/and CD8+ T-cells were observed in all the 22

convalescent HCWs (100%), in 16 cases (73%) with cellular response to peptide S, in 17 (77%) toward M, and in 9 cases (41%) toward peptide N, without differences in response according to positive or negative RT-PCR. A T-cell response to 2 or more viral epitopes was observed in 17 cases (77%), predominantly against proteins S and M. Notably, the 6 HCWs with negative serology at this moment have a weaker T cell response in magnitude and breadth (**Supplementary Figure S1AB).**

At the end of follow up, after a median time of 155 days (IQR,141-162), 3 additional HCWs have lost their previous humoral response (total, 9 out of 22, 41%), and a significant decline in ELISA antibodies titer was observed in 77% of cases ($p<0.01$; **Figure 1A).** Thus, seroreversion was observed for those with the initial lowest ELISA titers of antibodies (24.8 vs 11.1; p=0.001).

We were able to repeat the evaluation of T-cell response in 16 HCWs. Five (31%) were not reactive, and there was a variation in the breadth and magnitude of SARS-CoV-2-specific responses (**Figure 1B-1C**). Of note, 9 out of 12 HCWs have lost T cell response to protein S. There was a significant association between humoral and T-cell response: loss of any T-cell response was observed in young HCWs who had a less severe disease (84% asymptomatic), with lower level of antibodies at inclusion (p=0.005) and at the end of follow up (p=0.019).

To test the impact of the initial immunological response, we evaluated 10 pro- and anti-inflammatory cytokines at inclusion. An increased production of IFN-γ, IL-2, and IL-6 against protein S, and up-regulation of IFN-γ, TNF-α, GM-CSF, IL-17, IL-2 and IL-6 and of IL-10 against protein M were significantly associated with persistent humoral response (**Figure 2**). Thus, an increased Th1 response against viral peptides was associated with persistence of specific antibodies against SARS-CoV-2 (p=0.017).

During an median additional follow up of 116 days (IQR, 96-131), 5 HCWs were studied because of suggestive symptoms (sore throat, headache) or close contact with COVID-19

patients/partner, without any demonstrated case of reinfection, despite the fact that most of them continued working on the frontline of COVID-19.

Discussion

The durability of infection-induced SARS-CoV-2 immunity has major implications for estimating the risk of reinfection of HCWs and vaccine expectations. We measured antibodies and T-cell responses in paired samples obtained an average of 1.8 and 5.1 months after infection, showing a close correlation between humoral and specific T-cell response and the importance of the initial immune response for predicting the duration of this response.

Although SARS-CoV-2 elicit a robust B cell response, cumulative evidence showed a loss of specific humoral response to SARS-CoV-2 after disease, that ranged from 14 to 50% of initially positive patients [2, 5]. In our study, we found that 27% of convalescent HCWs have lost specific antibodies as early as 2 months, a rate that increased to 41% in a short follow up. In line with this, a recent study referred a constant decline in the level of antibodies in up to 94% of HCWs [6].

Nevertheless, it was unclear whether this decrease in SARS-CoV-2 antibodies represent the normal evolution following pathogen clearance[7], with maintenance of protective T-cell mediated responses. Nevertheless, we observed a relation between initial humoral and T cell response, and a weak cellular response was observed in those with early loss of antibodies, as previously reported [3]. Moreover, we also observed a decline in the rate of T-cell response during follow up, highlighting the loss of response to protein S in 75% of cases. Previous studies demonstrate that the factors involved with a shorter duration of humoral response were a younger age, lower severity of disease, or a lower initial viral load[2, 8]. It has been hypothesized that the failure of the early/innate immune control makes necessary a larger adaptive response as seen in more severe cases of influenza [9]: a mild-moderate disease could produce a greater systemic response than that observed in asymptomatic cases, and such individuals appear to mount a more vigorous T-cell response. In line with

this, a recent study found that the lack of response in asymptomatic patients is due to a higher frequency of NK cells and early and transient increase of specific IgA, highlighting the role of the innate response[10].

We confirmed the importance of the adaptative immune response. It has been shown that asymptomatic individuals had a reduced inflammatory response characterized by low circulating concentrations of cytokines and chemokines[2]. Our analysis of cytokines release after cell stimulation revealed up-regulation of pro- and anti-inflammatory cytokines in mild-moderate disease in comparison with asymptomatic, with an adequate Th1 in those maintaining both humoral and T cell response [11]. Indeed, current evidences strongly indicated that Th1 type response is a key for successful control of SARS-CoV-2. Furthermore, we observed an initial response significantly greater to peptide M, a peptide with 90% of structural homology between different coronavirus [12], suggesting the possible protective role of a background of cross-reactive immunity as observed in unexposed individuals[13, 14].

We did not observe any case of reinfection in our small cohort. As hypothesis, the failure to detect T cell response does not rule out the capacity of eliciting an immune response, which could be sufficient to control viral replication efficiently. Nevertheless, we consider that we do not have enough sample size and systematic follow up to be able to correctly assess this question.

Our study has several limitations, including the small sample of convalescent HCWs. A false positive serologic result in those with negative RT-PCR was possible but unlikely, because of the high sensitivity of repeated serological test. Indeed, a similar initial T-cell response in breadth and magnitude was observed in these participants, in comparison with RT-PCR positive individuals. Also, we quantified cytokines in PBMCs after stimulation, therefore produced by different immune cells, trying to mimic the overall response in vivo, an approach recently evaluated in SARS-CoV-2 infected patients[15]. Finally, we determine T cell

response to 3 structural viral proteins, and not include other viral immunodominant epitopes as ORF1, limiting our vision of the breadth of cellular immune response.

In conclusion, we found a close and parallel relation between humoral and cellular immune response to SARS CoV-2 in convalescent HCWs with asymptomatic or mild disease. Whereas age and severity are clinical factors, the development of a well-balanced adaptative immune response is responsible for the magnitude and duration of response. In any case, more research is needed to clarify the mechanisms underlying immune responses across the spectrum of clinical presentations and to evaluate the durability of immunity elicited by primary SARS-CoV-2 infection or vaccination.

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Author contributions

Jose L Casado and Alejandro Vallejo contributed equally to this work. JC and AV conceived of and designed the study, analyzed the results, and wrote the manuscript; HV conducted the study and performed analytical determinations; PV, JH, AMcG, MFE conducted the study and collected data, revised the manuscript, and approved the final version.

Data sharing

Requests for materials or data should be addressed to corresponding authors upon request.

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Figure 1.

A) Presence of specific antibodies against SARS-CoV-2 (ELISA): change in the plasma ELISA titers between the baseline visit and follow-up visit. Lines between points indicate individual changes for the 22 HCWs involved. Values above discontinued line (11 RU) indicate positivity.

B) IFN-γ producing CD8+ and CD4+ T-cell (%) in HCWs at inclusion (black circles), and at the end of the study (grey squares) in December 2020. SARS-CoV-2-specific CD8+ and CD4+ T cells (%) were calculated as the sum of the S-, M-, and N-specific CD8+ and CD4+ T cells. Each dot represents a different individual. Lines indicate median.

C) Changes in the breadth of response to viral peptides from the baseline visit (black bars) to the end of the study (grey bars). Changes in the percentage of individuals with CD8+ and CD4+ T-cell response toward different structural viral proteins (S, spike; M, membrane; N, nucleocapsid). Legend: SMN, response to the three viral proteins; S+, presence of response to S (+ to another protein); M+, response to M and to another protein; N+, response to N and to another protein.

Figure 2. Differences in specific cytokines (IFN-γ, IL-2, IL-6, IL-10) response in PBMCs after stimulation with different structural viral peptides (S, circles; M, triangles; N, diamonds), according to the persistence of humoral response (IgG positive, black; IgG negative, grey) at the end of the study. Each dot represents a different individual. Lines indicate median.

Figure 1A.

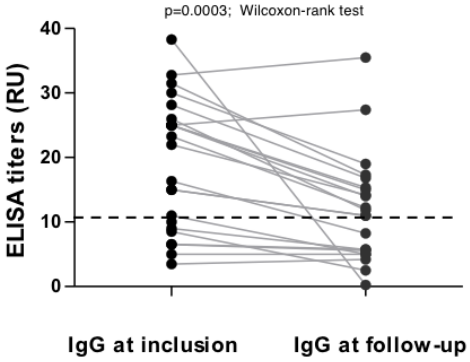
p=0.0003; Wilcoxon-rank test
ELISA titers (RU)
40
30
20
10
0
IgG at inclusion
IgG at follow-up

Figure 1B

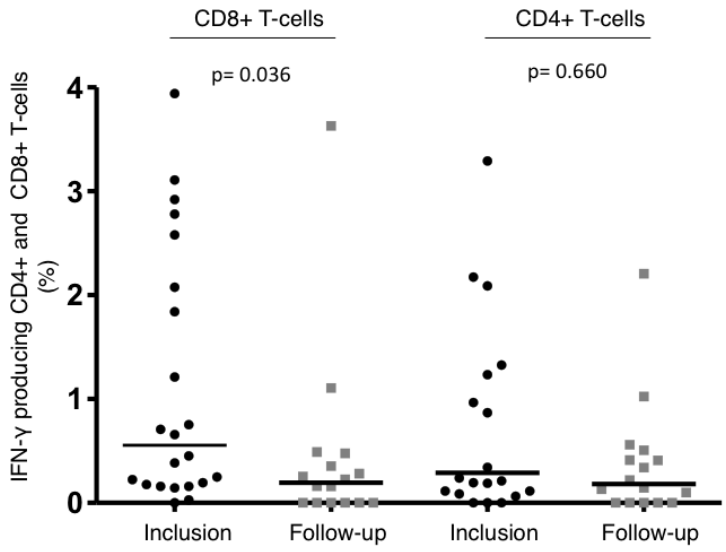

Figure 1C

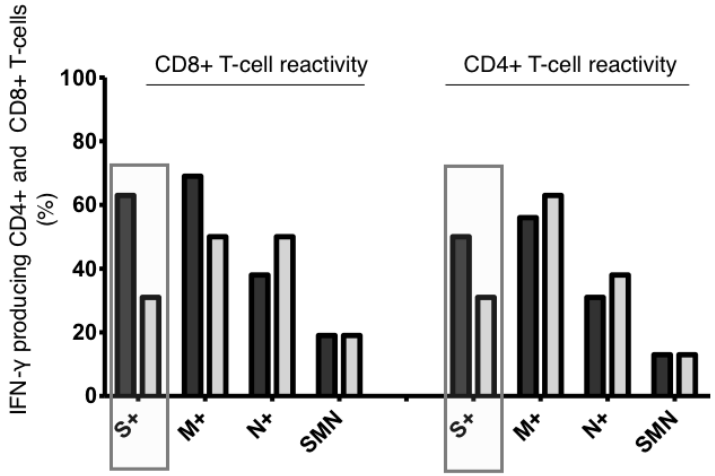

Figure 2