ASSESSMENT OF SARS-COV-2 SPECIFIC B-CELL IMMUNE MEMORY: EVIDENCE FOR PERSISTENCE UP TO 1 YEAR POST-INFECTION

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ABSTRACT

Background: SARS-CoV-2, the virus responsible for COVID-19 pandemic, has posed huge global health challenges. Understanding the immune response to SARS-CoV-2 infection, and in particular – the role of B cells in the generation of immune memory is crucial for assessing the durability of protective immunity.

Materials and Methods: In this longitudinal prospective study, individuals who had recovered from SARS-CoV-2 infection were included. Peripheral venous blood samples were collected at three time intervals post symptom onset (PSO): 1-3 mo, 4-8 mo, and 9-12 mo. The humoral immune response was evaluated by measuring anti-SARS-CoV-2 IgG, virus-neutralizing antibody activity, total S1-specific B-cells, and B cell subpopulations.

Results: The levels of anti-SARS-CoV-2 specific IgG antibodies decreased from 390.3 to 204.5 BAU/ml in the first 6-8 months PSO but did not significantly decrease further until the 12th mo (126.6 BAU/ml). Virus-neutralizing antibodies (activity decreased by 20.4% between the 1st and 6-8th mos but remained relatively stable thereafter and could be detected up to 12 months PSO. In peripheral blood, the amount of S1-specific plasmablasts was highest one month after COVID-19 infection, and the level of memory B cells at 6 months. Those were detected even 12 months PSO, albeit in smaller quantities.

Conclusion: The study provides evidence for the persistence of SARS-CoV-2-specific B-cell immune memory up to 1 year post-infection. The presence of virus-specific memory B cells and plasmablasts suggests potential for sustained protection against reinfection. Further research is needed to elucidate the role of B-cell immune memory in preventing infection and to understand the individual variations of immune response.

Key words: SARS-CoV-2, Immune memory, Humoral immunity, B-cell immunity

INTRODUCTION

SARS-CoV-2, the novel coronavirus responsible for COVID-19 pandemic, has posed a significant global health challenge (1). Understanding the nature of immune response induced by SARS-CoV-2 infection is crucial for describing the dynamics of protective immunity and development of public health strategies. Among the key components of immune response, B cells play a vital role in combating viral infections by producing specific antibodies and generating immune memory.

One essential aspect of B cell immune memory is the duration of antibody production and the persistence of circulating protective antibodies following recovery from SARS-CoV-2 infection (2,3). Understanding the longevity of B cell immune memory is important for assessing the risk of reinfection and the effectiveness of acquired immunity against subsequent exposures to the virus (4). Several studies showed that after the acute phase of SARS-CoV-2 infection, there is an initial rapid elevation of antibody levels. However, the antibody levels tend to decrease over time reaching a plateau 8 months PSO, and gradually declining thereafter (5). This is a normal dynamic of the immune response.
response, and it does not necessarily indicate a loss of immune memory. Instead, it suggests a transition from short-lived plasma cells to long-lived memory B cells that can persist in the body for an extended period. (6)

The generation of virus-specific B-cell immune memory involves the formation of germinal centers within the secondary lymphoid organs, where B cells undergo clonal expansion, affinity maturation, and class-switching. These processes contribute to the production of high-affinity antibodies with enhanced neutralizing capacity. Moreover, memory B cells can persist in circulation or reside in specialized niches, poised to respond promptly and effectively upon re-exposure to virus (7). Several publications indicated that memory B cells generated in response to SARS-CoV-2 infection can persist for several months after recovery (9,10) and even up to one year post-infection (11,12,13).

In this study, we demonstrated that one year PSO S1-binding and neutralizing antibodies along with S1-specific B cells and plasma cells were detected in the circulation. This supports the hypothesis that SARS-CoV-2 infection induces durable humoral immunity.

MATERIALS AND METHODS
This longitudinal prospective study included individuals recovered from SARS-CoV-2 infection, confirmed with RT-PCR. The participants were aged between 18 and 75 years. Peripheral venous blood samples for isolation of peripheral blood mononuclear cells (PBMCs) and serum were taken and analyzed at three-time intervals: (1-3 mos, 4-8 mos, and 9-12 mos PSO). In 20% of the cases, the analysis was performed on different patients in the mentioned time periods. (Table 1).

The amount of anti-SARS-CoV-2 IgG binding antibodies was assessed by ELFA (Enzyme-linked fluorescent assay, VIDAS PC) and expressed as BAU/ml (Binding antibody units per mL). The presence of SARS-CoV-2-neutralizing antibodies was measured by the percentage of inhibition in a surrogate virus neutralization test (sVNT, GenScript kit).

Total S1-specific B cells were evaluated by B-ELISpot (Mabtech). B-ELISpot test was performed after a preliminary stimulation of $2 \times 10^6$ PBMCs with IL-2 and R848 for 5 days. The number of SARS-CoV-2-specific IgG-secreting B cells was measured as spot-forming units (SFU) per million PBMCs (SFU/10^6 PBMC) using an automated system – BIOREADER 700.

Multiparameter flow cytometry of PBMCs was used to identify peripheral blood S1-specific memory B cells. Based on the expression of specific CD markers, we developed a 13-parameter panel for the analysis of B-cell subpopulations: CD45 (BUV496), CD24 (BV421), CD27 (BV480), CD19 (BV605), CD20 (BV786), CD38 (PE), IgD (BB515), IgG (PE-Cy7), IgM (APC), CD138 (PE-CF594), CD21 (BUV395), Streptavidin (APC-R700), Streptavidin (BV605). To exclude T cells, NK cells, and monocytes from the analysis we used CD3, CD14, CD16 and CD56 mAbs stained with the same fluorochrome (PerCP/Cy5.5).

Antigen-specific B cells were identified using tetramers of four biotinylated S1 proteins and streptavidin conjugated with two different fluorochromes (BV421 and BV711). They were added to the CD marker panel along with streptavidin labeled with a third fluorochrome (BUV395 – decoy streptavidin). Stained cells were collected by FACSAria III flow cytometer and analyzed with DIVA v.8 software. The cells which were simultaneously stained by both tetramers and not stained by decoy streptavidin, were considered antigen-specific. An algorithm was created for the analysis of B-cell subpopulations and the identification of SARS-CoV-2-specific memory B cells and plasmablasts.

Statistical analysis was performed by GraphPad Prizm 8.0.1 and IBM SPSS 28.0. Data were presented as mean values ± SD. P was considered statistically significant at p<0.05.

RESULTS
Levels of S1 binding and virus-neutralizing antibodies.

The results for anti-SARS-CoV-2 IgG binding antibodies are presented in Figure 1A. The mean levels of anti-

<table>
<thead>
<tr>
<th>Testing period (mos PSO)</th>
<th>Male (n)</th>
<th>Female (n)</th>
<th>Age (years, X±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>20</td>
<td>46</td>
<td>44.5±22.5</td>
</tr>
<tr>
<td>4-8</td>
<td>7</td>
<td>41</td>
<td>45.5±21.5</td>
</tr>
<tr>
<td>9-12</td>
<td>14</td>
<td>19</td>
<td>49.5±22.5</td>
</tr>
</tbody>
</table>
SARS-CoV-2 IgG decreased significantly during the first 6-8 mos PSO from 390.3 to 204.5 BAU/ml (p<0.001). Afterwards, no additional significant decrease was observed up to the 12th mo PSO [126.6 BAU/ml (p>0.05)]. The levels of virus-neutralizing antibodies decreased by 20.4% between 1st and 6-8 mos after recovery (from 79.9% to 63.6% inhibition, p<0.001), followed by insignificant changes up to one year, (59.8% inhibition, p>0.05). Thus, a virus-neutralizing activity could still be detected up to 12 mos after the last contact with the viral antigens (Figure 1B).

**S1-specific memory B-cells evaluated by B-ELISpot**

During the first period (1-3 mos PSO), an average of 143.5±59 SFCs (Spot-forming cells) per 10⁶ PBMCs were detected. During the second period (4-8 mos PSO), the number of S1-specific B-cells significantly decreased (11.4±13.8, p<0.05), and at the end of the first year (9-12 mos PSO), an insignificant increase was registered (47.5.3±40.7, p>0.05). These results are presented in Figure 2.

**Memory B-cell subpopulations evaluated by flow cytometry**

The results of the peripheral blood B-cell subset analysis are presented in Table 2. The percentages of naive, transitional, and memory B cells did not show significant differences between the three studied periods. As might be expected, the percentages of plasmablasts and plasma cells were highest 1-3 mos PSO (6.3% and 6.9%, respectively). The share of these subpopulations decreased twice at the end of the first year (2.9%, and 3.2%, respectively, p<0.05 for both). The average share of NCSMB (non-class-switched memory B cells) increased between the first (1-3 mos) and second period (9-12 mos) from 39.2% to 59.4% (p<0.05). In parallel, the percentage of CSMB (class-switched memory B cells) decreased insignificantly during the same time, from 30.5% to 26.3% (p>0.05).

**Antigen-specific B-cells and plasmablasts evaluated by flow cytometry.**

The results about virus-specific B cells and plasmablasts are presented in Figure 3. During the first period (1–3 mos PSO) the percentage of S1-specific CSMB exceeded that of NCSMB (0.6% vs 0.2%, p<0.05). The amount of CSMB remained stable until 6 mo and decreased threefold afterwards (0.7% vs 0.2%, p<0.05). The level of NCSMB was low during the first period (1–3-mos PSO) (0.2%), followed by a 5-fold increase between 4-8 mos, (1.3%, p<0.05), and a subsequent decrease

<table>
<thead>
<tr>
<th>Table 1. Percentage distribution of B cell subpopulations.</th>
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<tbody>
<tr>
<td>B cell subset (phenotype)</td>
</tr>
<tr>
<td>Naive B cells</td>
</tr>
<tr>
<td>CD45⁺/CD19⁺/CD20⁺/CD21⁺/CD24⁺/IgD⁺/IgM⁺</td>
</tr>
<tr>
<td>Transitional B cells</td>
</tr>
<tr>
<td>CD45⁺/CD19⁺/CD20⁺/CD21⁺/CD24⁺/CD38⁺⁺⁺/IgD⁺</td>
</tr>
<tr>
<td>Memory B cells</td>
</tr>
<tr>
<td>CD45⁺/CD19⁺/CD20⁺/CD21⁺/CD27⁺/CD24⁺</td>
</tr>
<tr>
<td>NCSMB (% from memory B cells)</td>
</tr>
<tr>
<td>CD45⁺/CD19⁺/CD20⁺/CD21⁺/CD27⁺/CD24⁺/IgD⁻/IgM⁺/IgG⁻</td>
</tr>
<tr>
<td>CSMB (% from memory B cells)</td>
</tr>
<tr>
<td>CD45⁺, CD19⁺, CD20⁺, CD21⁺, CD27⁺, CD24⁺, IgD-, IgM⁻low, IgG⁺</td>
</tr>
<tr>
<td>Plasmablasts</td>
</tr>
<tr>
<td>CD45⁺/CD19⁺/CD20⁻low/CD27⁻/CD38⁻/⁺⁺/CD138⁻⁺⁺, IgD⁻low/ IgM⁻low</td>
</tr>
<tr>
<td>Plasma cells</td>
</tr>
<tr>
<td>CD45⁺, CD19⁺, CD20⁻low, CD27⁺, CD38⁺⁺, CD138⁻⁺⁺, IgD⁻low, IgM⁻low</td>
</tr>
</tbody>
</table>
between 9-12 mos to 0.3% (p<0.05). The share of S1-specific plasmablasts in peripheral blood was highest between 1 and 3 mos PSO (4%). During the second period (4-8 months PSO) plasmablasts decreased to 0.7% (p<0.05), followed by a new increase to 3.8% between 9 and 12 mos (p<0.05).

**DISCUSSION**

In this study, we investigated the duration of humoral immunity after recovery from COVID-19 according to the following parameters: S1-binding and neutralizing antibodies, S1-specific total memory B cells, as well as their subpopulations. We found that SARS-CoV-2 infection induced the formation of S1-binding and neutralizing antibodies. Their levels were highest during the first 3 months after recovery. Although their level decreased later, it was still detectable until the end of the first year PSO. This is consistent with the results of other authors and supports the notion that SARS-CoV-2 likely builds long-lasting immunity. To answer the question about the longevity of B cell immunity, we investigated the circulating total S1-specific memory B cells as well as their subpopulations. We found that the amount of total S1-specific memory B cells in the blood was highest 4-8 mos PSO, followed by a decrease. However, during the third period (9–12 mos PSO), a significant amount of S1-specific total memory B cells were found in circulation. These results are in line with other authors’ published data (13-17). The presence of S1-B memory cells one year after infection supports the hypothesis of lasting B-cell immune memory.

The results presented in Figure 1 showed a nonlinear reduction of S1-specific total memory B cells, measured by B-ELISpot. Therefore, we investigated the B cell subpopulations in more details by multiparameter flow cytometry. The six B-cell subpopulations showed different dynamics during the studied PSO periods. The percentage of CSMB cells followed the dynamics of serum antibodies, maintaining the level until 6 mos and decreasing until

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**Figure 1.** Dynamics of A) S1-specific anti-SARS-CoV-2 IgG and B) percentage of inhibition of virus-neutralizing antibodies.

**Figure 2.** Number of S1-SARS-CoV-2-specific plasma cells detected by B-ELISpot

**Figure 3.** Analysis of flow cytometric data for specific memory B cells and plasmablasts. (1)
the 12th mo. The amount of NCSMB cells increased between the first and second period (4–6 mos PSO) of the study, followed by a decrease. The dynamics of S1-specific plasmablasts coincided with that of the total S1 memory cells detected by B-ELISpot. The presence of S1-specific plasmablasts in the circulation after recovery from COVID-19 was also described by other authors (18). In this study, at 9-12 mos PSO we detected also S1-CSMB and S1-NCSMB cells in the peripheral blood. This finding gives us the reason to assume that following SARS-CoV-2 infection, B-cell immune memory dynamically develops for at least 12 mos, which is the basis for establishment of long-lasting immunity. Further research is needed to establish the longevity of this immune memory and its protective effect.

**CONCLUSION**

Our results show that using S1 tetramers and multiparameter flow cytometry, virus-specific B-cell immune memory can be assessed in detail. S1-specific plasmablasts and memory B cells were detected by B-ELISpot and S1 tetramers 12 mos PSO albeit in small amounts. This finding supports the hypothesis that long-lasting B-cell immune memory is possible after COVID-19, although its protective role remains to be clarified.

**LIMITATIONS**

A major limitation of the study is the decreased number of tested individuals during the subsequent periods of testing, due to reinfection or vaccination of the original donors. Another limitation is the fact that in 20% of the cases, the analysis in the studied period of testing, due to reinfection or vaccination of the original donors. Another limitation is the fact that in 20% of the cases, the analysis in the studied time periods was performed on different patients.

**ACKNOWLEDGMENT**

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