Immunisation with Coronavirus-2 vaccines induces potent antibody responses and does not aggravate the lymphocyte compartment of primary Sjögren's syndrome patients

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Abstract

Objective

The peripheral lymphocyte compartment of patients with primary Sjögren's syndrome (pSS) differs strongly from healthy individuals. Whether this altered lymphocyte composition also changes abnormally during immune reactions, especially by novel CoV-2-vaccines, is unknown.

Methods

Peripheral blood mononuclear cells (PBMC) from 26 pSS patients and 6 healthy controls were compared before Coronavirus-2 (CoV-2) vaccination (Pfizer/BNT162b2, Moderna/mRNA-1273, AstraZeneca/AZD122 ChAdOx1 nCoV-19) and 7 days after secondary vaccination. Spike 1 (S1)-receptor binding domain (RBD)-specific IgG antibodies were measured in serum samples. Among PBMCs, B and T cell subpopulations were phenotypically analysed and RBD-specific B and plasma cells were evaluated.

Results

Immunisation induced CoV-2 specific serum antibodies in all pSS patients and healthy participants. When analysing pSS patients and controls together, frequencies of circulating IgG+ RBD-specific antibody-secreting cells (ASC) and anti-RBD serum titres correlated (r=0.42, p=0.022). Previously described alterations of peripheral B cells in pSS patients (e.g. reduced memory B cells, increased naive and transitional B cells and higher maturity of ASCs) remained stable during vaccination. The subset distribution of CD4+ and CD8+ T cells also stayed largely unchanged. However, frequencies of CD4+CXCR5-PD-1+ circulating peripheral helper T (cT_{PH})-like cells increased in pSS patients comparing pre- and post-vaccination (p=0.020), while circulating CD4+CXCR5+PD-1+ follicular helper T (cT_{FH})-like cells declined (p=0.024).

Conclusion

An immune reaction induced by vaccination with the novel CoV-2 vaccines yields adequate antibody production and vaccine specific lymphocytes in pSS patients and controls. Aberrant lymphocyte subset distribution in pSS patients persisted after vaccination and no major changes were induced despite small changes in cT_{PH} and cT_{FH} cells.

Key words

Sjögren's syndrome, vaccination, Coronavirus-2, lymphocytes

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Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease with local inflammation predominantly in lacrimal and salivary glands (1). Peripheral blood of pSS patients has a unique lymphocyte signature with decreased CD27+ memory B cell (B_{MEM}) but increased naive B cell, transitional B cell and plasmablast frequencies (1, 2). Peripheral CD4+ T cell frequencies are lower in pSS patients and shift from naive (CD45RA+CCR7+) and central memory (CD45RA-CCR7+) towards effector memory (CD45RA-CCR7-) and CD45RA+CCR7- effector memory (TEMRA) phenotypes (3). In line with B cell hyperactivity, pSS patients are characterised by increased circulating peripheral (cT_{PH}) and follicular (cT_{FH}) helper T cells (3).

Previous studies addressed vaccine responses in pSS patients using seasonal influenza (H1N1) vaccination. pSS patients exhibited a higher anti-H1N1 response than controls with simultaneously increasing anti-EBV, anti-SSA/Ro and anti-SSB/La titres without development of disease flares (4, 5). Total plasmablasts and B_{MEM} frequencies remained stable after influenza vaccination in pSS and healthy individuals. Circulating cT_{FH} cells expanded in healthy controls but not in pSS patients post-vaccination (6).

We recently reported no difference in anti-receptor binding domain (RBD) antibodies or the T cell interferon γ-response in pSS patients compared to controls suggesting vaccine efficacy also in pSS patients (7). Disease activity and anti-SSA/Ro titres remained unchanged post-CoV-2 vaccination and side effects were comparable to controls (7).

The lymphocyte compartment in pSS patients is fundamentally different compared to healthy individuals and an immune stimulus by vaccination might affect its composition differently compared to controls (5, 6). Therefore, we performed detailed B and T cell phenotyping pre- and post-vaccination to investigate the effect of CoV-2 vaccination on the unique lymphocyte composition in pSS patients also in the context of novel state-of-the-art vaccines.

Material and methods

Study design

Patient in- and exclusion criteria have been previously described (7). Briefly, pSS patients fulfilled 2016 ACR/EULAR criteria, were SARS-CoV-2 naive, and were allowed to use only hydroxychloroquine or <10 mg/day prednisone. Participants were vaccinated according to the Dutch vaccination programme with Pfizer/BNT162b2, Moderna/mRNA-1273 or AstraZeneca/AZD1222 vaccines. Mean time interval between vaccinations was 35±2 days for mRNA vaccines and 70±4 days for AZD1222, as approved by European health authorities. Blood samples were taken pre-vaccination (T1) and 7 days after second vaccination (T2). CoV-2 antibody titres were measured as described previously (7). Ethical approval was obtained from the University Medical Center Groningen (UMCG) institutional review board (METc 2014/491, METc 2021/084) and all participants provided written informed consent.

Spectral flow cytometry

Cryopreserved PBMCs were stained either with a B cell panel (Supplementary Table S1) or a T cell panel (Suppl. Table S2). The staining procedure comprised the following steps: 1. Fc Block, 2. surface staining, 3. viability staining, 4. fixation/permeabilisation and 5. intracellular staining (Suppl. Table S1, S2). An Aurora 5L spectral analyser (Cytek Bioscience, USA) was used for acquisition and FlowJo software 10.8 (Tree Star, USA) for analysis following the depicted gating strategy (Suppl. Fig. S1).

FlowJo software 10.8 was used to analyse T cells via tSNE dimension reduction. Viable CD3+ T cells were down-sampled to 6.000 cells per sample and both participant groups and timepoints were concatenated (64 samples, 384.000 total events). The FlowJo tSNE algorithm was used with 5.000 iterations and 100 perplexities.

Statistical analysis

Group comparisons were tested with Mann-Whitney U-test or Wilcoxon rank test dependent on data pairing. Ig isotype and T cell subset distribution were tested with 2-way ANOVA. Correla-

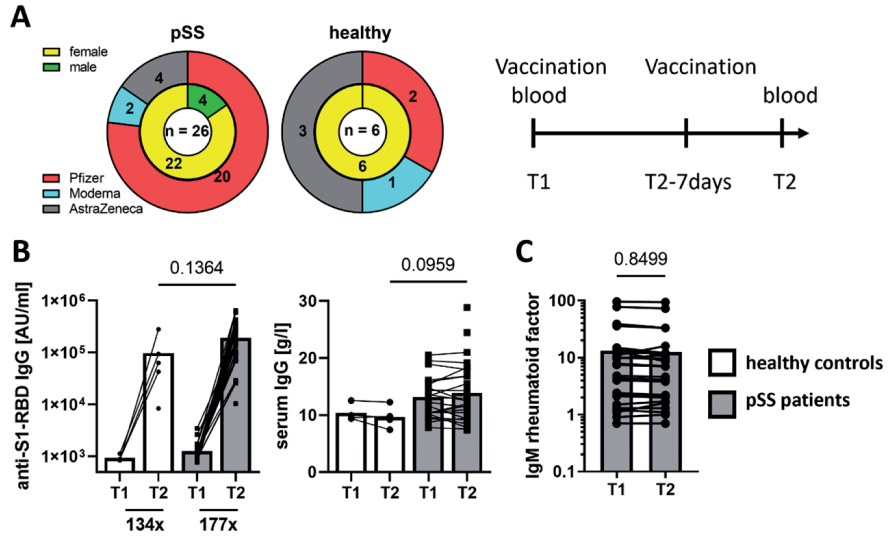

Fig. 1. pSS patients mount a normal humoral anti-CoV-2 response.
A: Gender and vaccine usage within the subset of the VaccineSS study cohort and timeline of the study sampling.
B: Anti-S1-RBD CoV-2 titres and serum IgG of pSS patients and healthy controls. Numbers below the graph indicate mean fold change of RBD titres between both sampled timepoints.
C: Serum titres of IgM rheumatoid factor in pSS patients. Bar charts with individual data points; paired values connected by lines; *p*-values determined by Mann-Whitney U-test or Wilcoxon rank test according to pairing of data.

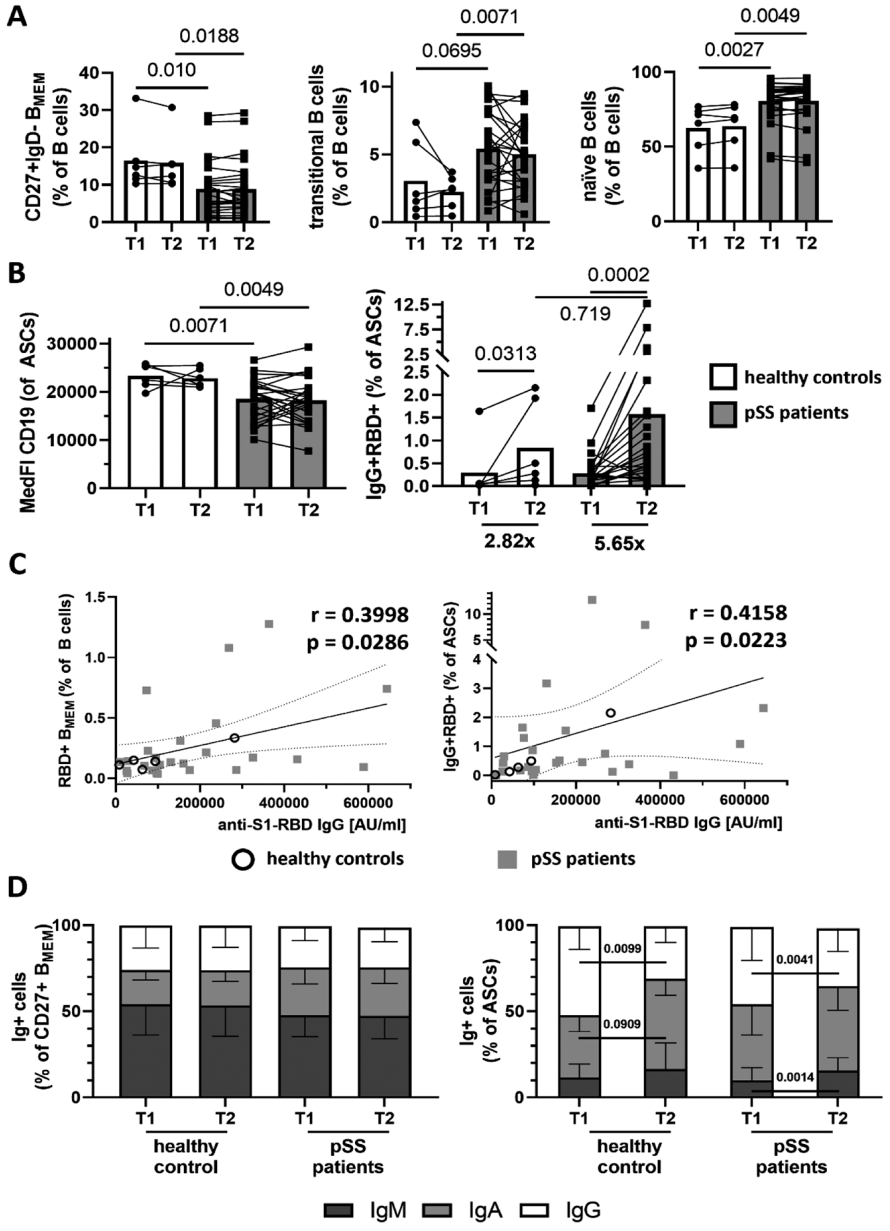

Fig. 2. B cell subpopulations remain stable in pSS patients after vaccination.
A: Frequencies of CD27+IgD- B_{MEM}, naive B and transitional B cells pre- and post-vaccination.
B: Median fluorescence intensity (MedFI) of CD19 on ASCs and percentage of IgG+RBD+ ASCs in pSS patients and healthy individuals at both study timepoints. Numbers below the graph indicate mean fold change between timepoints.
C: Correlation of RBD+ B_{MEM} cells and IgG+RBD+ ASCs with anti-S1-RBD CoV-2 titres after second vaccination with combined data of pSS patients (grey square) and healthy controls (open circle).
D: Isotype distribution of CD27+ B_{MEM} and ASCs in pSS and control groups.
Bar charts with mean and individual data points; paired values connected by lines; *p*-values determined by Mann-Whitney U-test or Wilcoxon rank test according to pairing of data. Scatter plots contain linear fit (solid line) with 95% confidence bands (dotted lines); *p*- and r-values calculated with Spearman correlation. Stacked bars with mean ± standard deviation; *p*-values calculated with 2-way ANOVA.

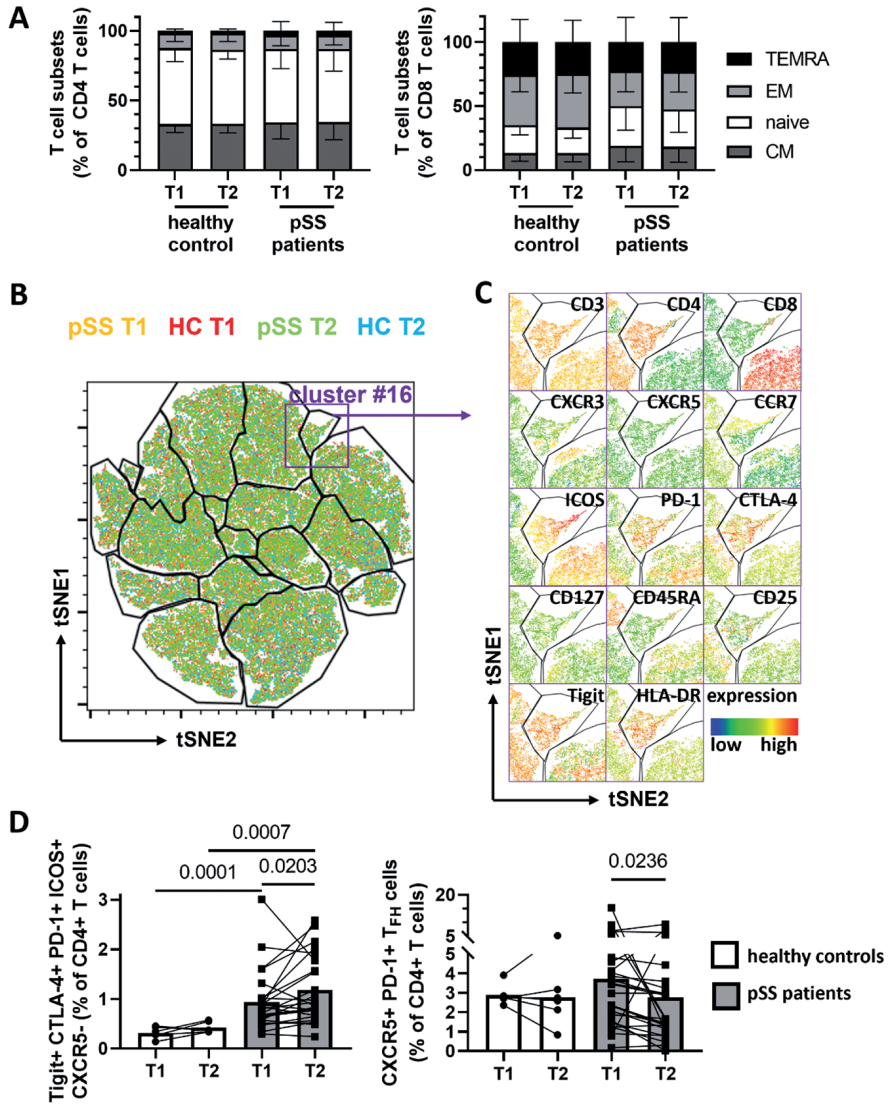

Fig. 3. CoV-2 vaccination change no major T cell subset, but T helper populations.
A: Subset distribution of CD4+ and CD8+ T cells into CCR7+CD45RA+ naive, CCR7-CD45RA+ TEMRA, CCR7+CD45RA- central memory (CM) and CCR7-CD45RA- effector memory (EM) cells.
B: tSNE plot of T cells combined from pSS patients and healthy control (HC) samples from pre- and post-vaccination timepoints indicated by colour.
C: Detailed view of cluster #16 showing expression of T cell markers used for flow cytometry phenotyping.
D: Frequencies of circulating CD4+PD-1+ T helper populations separated in CXCR5- cT_{PH} like cells and CXCR5+ cT_{FH} cells in healthy controls and pSS patients.
Bar charts with mean and individual data points; paired values connected by lines; *p*-values determined by Mann-Whitney U-test or Wilcoxon rank test according to pairing of data. Stacked bars with mean ± standard deviation; *p*-values calculated with 2-way ANOVA.

tions were tested using Spearman's correlation. Calculations were performed with GraphPad Prism software 9.2.0.

Results

Patients with pSS mount a potent vaccination response

Patients with pSS (n=26; mean age 58.7±12 years) were vaccinated against CoV-2 either with Pfizer/BNT162b2 (n=20), Moderna/mRNA-1273 (n=2) or AZD1222 (n=4) vaccines, while healthy controls (n=6; mean age 48.5±15 years) received less Pfizer/BNT162b2 (n=2) and more Moderna/mRNA-1273 (n=1) or AZD1222 (n=3) vaccines (Fig. 1A). All participants mounted detectable anti-S1-RBD IgG responses, while total serum IgG remained stable after vaccination in both groups (Fig. 1B, Suppl. Fig. 2B). Similar to previously published anti-SSA titres (7), IgM-rheumatoid factor remained unchanged in pSS patients after vaccination (Fig. 1C).

B cell subpopulations remain stable in pSS patients after vaccination

Although the vaccination response appeared normal in pSS patients, we questioned whether B cell subpopulations (Fig. 2A, Suppl. 1A) were affected by vaccination. Total lymphocyte and B cell counts were comparable between pSS patients and healthy individuals for both timepoints (Suppl. Fig. 2A). As typical for peripheral B cells in pSS patients, CD27+IgD- B_{MEM} cells were decreased, while naive and transitional B cells were increased compared to healthy individuals pre-vaccination. Frequencies of these B cell subsets remained stable after immune stimulation by vaccination in pSS patients and healthy controls (Fig. 2A). Even at the individual sample level, vaccination-induced changes of memory and naive B cell frequencies were small. RBD+ B_{MEM} frequency among B cells was comparable in pSS patients and controls (0.27±0.33 *vs*. 0.16±0.07; p=0.98) post-vaccination.

Total antibody-secreting cell (ASC) counts increased similarly in pSS and healthy individuals after vaccination (Suppl. Fig. 2A). While maturity of ASCs, reflected by low CD19 expression, was unchanged in pSS and healthy individuals post-vaccination, pSS patient ASCs were more mature at both timepoints. Peripheral IgG+ ASCs recognising RBD were robustly induced in pSS patients and controls by vaccination (Fig. 2B). A correlation of anti-RBD serum titres with circulating RBD+ B_{MEM} cells and IgG+RBD+ ASCs was observed post-vaccination (Fig. 2C).

Additionally, we addressed the isotype distribution of circulating CD27+ B_{MEM} cells and ASCs. The isotype composition of B_{MEM} cells was similar comparing pSS patients and healthy individuals both pre- and post-vaccination (Fig. 2D). Compared to baseline, IgG+ ASCs were significantly lower after vaccination in pSS and healthy individuals in favour of IgM+ ASCs in pSS patients and IgA+ ASCs in healthy controls (Fig. 2D).

T helper cell populations shift from cT_{FH} to cT_{PH} cells in pSS patients

T cells are crucial for both humoral and cellular vaccine responses. Total T cell counts were comparable between groups and time points, but CD4 T cell numbers were lower in pSS patients at both time points, as expected (Suppl. Fig. 2A). No significant differences in naive (CCR7+CD45RA+), central memory (CM; CCR7+CD45RA-), effector memory (EM; CCR7-CD45RA-) nor TEMRA (CCR7-CD45RA+) CD4 and CD8 T cell subsets were found comparing pSS and healthy participants or pre- and post-vaccination timepoints (Fig. 3A, Suppl. Fig. 1B).

To investigate more detailed T cell subsets in pSS and healthy individuals, we analysed T cells from all individuals using a tSNE approach (Fig. 3B). Out of 19 identified clusters only cluster #16 was significantly enriched at baseline in pSS patients compared to controls. This subset further enriched in pSS patients post-vaccination. Cluster #16 contained CD4+ cells expressing Tigit, ICOS, PD-1 and CTLA-4 (Fig. 3C) but lacking CXCR5, thus mirroring the phenotype of circulating peripheral T helper cells (cT_{PH}; CXCR5-PD-1+). Manual gating confirmed higher frequencies of this T cell population in pSS patients compared to controls pre- and post-vaccination. cT_{PH} cells additionally increased in pSS patients after vaccination (Fig. 3D). By contrast, CXCR5+PD-1+ circulating follicular T helper cell (cT_{FH}) frequencies were comparable between pSS and healthy participants, but these cells decreased in pSS patients post-vaccination. cT_{PH} and cT_{FH} frequencies did not change in healthy controls after vaccination (Fig. 3D).

Discussion

The adaptive immune system of pSS patients features a pronounced disbalance and hosts auto-reactive and hyperactive B cells (8). Vaccines contain foreign antigen and are supposed to stimulate a specific immune reaction. A report on squalene-containing influenza vaccines showed elevated anti-SSA/SSB and anti-EBV levels in pSS patients post-vaccination (5). An anti-influenza vaccine without adjuvant hardly increased anti-SSA/SSB titres in pSS patients (4). Therefore, increasing serum anti-EBV and autoantibodies post-vaccination may be adjuvant-induced in pSS patients. In mice studies squalene-based emulsions are shown to cause tissue damage at the injection site (9) and induce auto-antibodies (10). In pSS patients, squalene-induced cell damage might cause activation of hyperactive (auto-reactive) lymphocytes. The CoV-2 mRNA vaccines Pfizer/BNT162b2 and Moderna/mRNA-1273 only contain lipid nanoparticles and CoV-2 spike mRNA, but no additional adjuvant (11), similarly to the AZD1222 vector vaccine. Anti-SSA (7) and rheumatoid factor levels remained stable in our study cohort, consistent with the previous findings concerning non-adjuvanted vaccines.

Here, we show that the major circulating B and T cell subpopulations, which are altered in pSS patients compared to healthy controls, remain stable during vaccination. Except an increase in cT_{PH} cells with simultaneously decreasing cT_{FH} cells is observed in pSS patients. In healthy individuals, CoV-2 vaccination or infection increases frequencies of cT_{PH} and cT_{FH} cells (12, 13). Both subsets remain stable in our healthy cohort, but the number of healthy participants is insufficient to draw definite conclusions. cT_{PH} and cT_{FH} cells have an overlapping differentiation mechanism, but cT_{PH} generation is fostered by chronic inflammation and higher interferon levels (14) both seen in pSS patients. Therefore, cT_{PH} generation may be favoured in pSS patients following an immune stimulus like CoV-2 vaccination.

Limitations of this study include low numbers of patients and controls, especially when analysed by the three included vaccines separately. Additionally, vaccinations were performed by the Dutch vaccination programme, leading to an uneven usage of vaccine types, which were also restricted to special requirements and age groups. Similar studies should be performed to confirm our observations in larger cohorts. The pSS patient group had predominantly low ESSDAI scores, but effects of the ESSDAI on anti-CoV2 titres were not observed in this cohort (7).

Together with our previous study reporting on CoV-2 vaccine effectiveness and safety in pSS patients (7), we demonstrate normal induction of vaccine specific ASC and B_{MEM} populations in pSS patients without altering their unique lymphocyte composition. This could indicate only B cell hyperactivity but not hyperimmune reactivity in pSS. In summary, these results argue for a save use of CoV-2 vaccines in pSS patients.

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