Anti-Spike Protein Antibody Immunoreactivity Is Widely Expressed in Human Lymph Nodes with or without Pfizer-BioNTech or Moderna mRNA Vaccination in Non-Infected Individuals

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Short Report

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Abstract

The novel coronavirus SARS-coronavirus 2 (SARS-CoV-2) is the cause of the coronavirus disease 19 (COVID-19), the rapidly spreading pandemic. When SARS-CoV-2 enters the target cell, the spike (S) glycoprotein binds to a cellular receptor angiotensin converting enzyme 2 (ACE2). Effective vaccination has been achieved, utilizing the Spike (S) protein mRNA sequence.

Objective: For a full understanding of the effects of Pfizer-BioNTech or Moderna mRNA vaccines, we evaluated the lymphoid responses. We have performed S protein western blots for proteins extracted from axillary lymph nodes, and S protein immunohistochemistry for the axillary lymph node tissues from human autopsies.

Results: Our results showed that both vaccinated and control cases (non-vaccinated or negative for anti-S and anti-N antibodies) had positive S protein reactivity in both western blots and immunohistochemistry. This reactivity was present several months after vaccination. One anti-S protein antibody western blots showed a positive correlation with serum anti-S protein amounts. The positivity in non-vaccinated uninfected individuals indicates that either the antibodies against S protein are cross-reacting to other proteins present in human tissues, or due to insidious infections to SARS-CoV-2. Further evaluation is necessary for the reliability of anti-S protein antibodies in SARS-CoV-2 studies.

Introduction

The novel coronavirus SARS-coronavirus 2 (SARS-CoV-2) is the cause of the coronavirus disease 19 (COVID-19), the rapidly spreading pandemic. When SARS-CoV-2 enters the target cell, the spike (S) glycoprotein binds to a cellular receptor angiotensin converting enzyme 2 (ACE2) \[^{1}\]. Effective vaccination has been achieved, utilizing the Spike (S) protein mRNA sequence \[^{2}\]. For a full understanding of the effects of vaccines, the lymphoid responses need to be evaluated. The Pfizer-BioNTech tozinameran/ famtozinameran and Moderna Spikevax original/ OmicronBA.1/ BA.4–5 vaccines are messenger RNA (mRNA) vaccines effectively preventing infection with SARS-CoV-2 \[^{3},^{4}\]. SARS-CoV-2 mRNA vaccines induce persistent human germinal center responses in draining lymph nodes \[^{5}\]. While the mRNA vaccines injected in muscles may produce S proteins in muscles and be presented to immune cells \[^{6}\], the potency of nanoparticle mRNA vaccines is tightly linked to their capacity to reach and accumulate in the lymph nodes draining the immunization site \[^{7}\]. The lipid nanoparticles used in mRNA vaccines may be transported by peripheral dendritic cells or by passive transport through afferent lymphatic drainage \[^{7}\]. The immune responses to mRNA vaccines coding S protein can be assessed immunologically by western blot and immunohistochemistry in axillary lymph node tissues, which are the draining site of deltoid muscle injections of mRNA vaccines. In this study, we assessed the western blot detection and immunohistochemistry of S protein in axillary lymph nodes in autopsy samples in correlation with vaccination history and serum anti-S protein IgG values.

Materials and methods
Materials

The human axillary lymph nodes (the right side in most cases) were obtained from forensic autopsies of our institution. The autopsy cases were Asian and shown in detail in Table S1 in Supplementary Files. The vaccination history of either Pfizer-BioNTech tozinameran/ famtozinameran or Moderna Spikevax original/ OmicronBA.1/ BA.4–5 vaccines was obtained wherever possible. At the time of autopsy, all cases were tested negative for nasal swab SARS-CoV-2 S protein antigen (ESPRINE SARS-CoV-2, Fujirebio, Tokyo, Japan), and negative for nasal swab SARS-CoV-2 N gene PCR (SARS-CoV-2 detection kit NCV-301_302, Toyobo, Osaka, Japan). Serum anti-S and anti-N antibodies (Elecsys Anti-SARS-CoV-2 RUO system, Roche Diagnostics, Tokyo, Japan) were measured at the time of autopsy [8]. The antibody test results are shown in Table S1 in Supplementary Files. The control cases (cases 1 through 5) were negative for the nasal swab S protein antigen and N gene PCR, negative for anti-S and anti-N antibodies, and had no known vaccination history. Among them, cases no. 4 and 5 had the confirmed history of no vaccination. Cases no. 6 through 11 had confirmed history of vaccination with elevated anti-S antibodies.

The study was approved by Teikyo University Ethical Review Board for Medical and Health Research Involving Human Subjects (Approve No. 21–118).

Anti-S Protein Antibodies used in this Study

The anti-S protein antibodies used in this study are ab272504 (rabbit polyclonal IgG, abcam, Cambridge, UK)[9–11], MAB105802 (mouse monoclonal IgG, R&D systems, Minneapolis, USA), MAB10890 (rabbit monoclonal IgG, R&D systems, Minneapolis, USA), GeneTex GTX635692 (rabbit monoclonal IgG, GeneTex, Irvine, USA)[12], GTX632604 (mouse monoclonal IgG, GeneTex) [13]. The second antibodies used for western blots are goat anti-rabbit IgG antibody conjugated to HRP, ab6721, Abcam, and goat anti-mouse IgG antibody conjugated to HRP, HAF007, R&D System. The titers for immunohistochemistry and western blots are shown in Table S2.

Western Blot Analysis

Western blots were used to quantify the S protein. The axillary lymph nodes were homogenized in lysis buffer (T-PER Tissue Protein Extraction Reagent with inhibitor, ThermoFischer Scientific, Waltham, USA), using POLYTRON (KINEMATICA, Switzerland). After the centrifuge at 10,000 G for 5 min, the supernatants were collected. Fifteen micrograms of protein per well were loaded and electrophoresed on 12.5% polyacrylamide gels, then transblotted to the PVDF membrane (ThermoFischer Scientific), using iBlot2 (ThermoFischer Scientific).

The first antibodies against SARS-Cov-2 Spike receptor binding domain (RBD), GTX635692, GTX632604, ab272504, MAB105802, and MAB10890 were applied to the PVDF membranes, using iBind Flex Western System (ThermoFischer Scientific), then the second antibodies (goat anti-rabbit IgG antibody conjugated to HRP, ab6721, or goat anti-mouse IgG antibody conjugated to HRP, HAF007) were applied. Then, SuperSignal West Pico PLUS Chemiluminescent Substrate (ThermoFisher Scientific) was applied, and
Luminescence was measured using Amersham Imager 680 (Cytiva, Tokyo, Japan). The loading amount of each lane in electrophoresis was standardized by the amount of GAPDH, measured by luminescence using anti-GAPDH antibody (anti-GAPDH rabbit IgG polyclonal antibody, 10494-1-AP, Proteintech, Rosemont, USA) and the above second antibody. The signal intensity of each western blot band was evaluated using ImageQuantTL (Cytiva, Tokyo, Japan).

Immunohistochemistry of Axillary Lymph Nodes

The autopsy tissues were fixed in 16% formalin, embedded in paraffin, and sectioned. The immunohistochemistry was performed by the BOND RX automated immunostainer (Leica, Germany) using BOND polymer refine detection system [14]. The 3 micrometer thick sections were pretreated with BOND Epitope Retrieval Solution. The anti-S protein antibodies used were ab272504, MAB105802, MAB10890, GTX635692, GTX632604. The second antibody was included in the BOND polymer refine detection system. Diaminobenzidine (DAB) was used for the staining. The immunohistochemical positivity was measured using cellSens software (Olympus, Tokyo, Japan). The numbers of DAB positive cells per x400 field were calculated using the cellSens software (Olympus). Within the lymph nodes, follicular areas, para follicular areas, medullary areas were separately counted.

Measurement of Serum Anti-S Protein and Anti-Nucleocapsid (N) Protein Antibodies

The anti-S protein and anti-N protein antibodies in the sera were measured by Roche Elecsys Anti-SARS-CoV-2 RUO system to evaluate the immunization state [8].

Statistical Analysis

Graph drawing, Mann Whitney tests and linear regressions were made by Prism 9 (GraphPad Software, USA). The p values less than 0.05 were considered statistically significant.

Results and Discussion

Western Blot Analysis with Anti-S Protein Antibodies

The western blot results for S protein immunoreactivity in proteins extracted from lymph nodes are shown in Fig. 1. Our study shows that the S protein immunoreactivity in western blots is present in vaccinated cases (case no. 6 through 11). The band sizes are consistent with molecular weights of cleavage products of S protein [15]. Cases no. 6 and 7 show positive more than 1 year after the last vaccine jabs, which is consistent with one previous report by Cognetti [16]. Cognetti et al reported that S protein rapidly increases after each vaccine dose and decreases back to baseline within 1–2 weeks, however, one case in their study showed the presence of serum S protein at about 50 days after the second dose [16]. Our result is consistent with Cognetti’s result in that S protein persists over months in the body.
On the other hand, western blot immunoreactivity against S proteins is also present in control cases where either no S protein mRNA vaccination was performed, or no existence of previous COVID-19 infection, assumed from the absence of anti-S and anti-N protein antibodies in the sera (Fig. 1). Five antibodies used in this study recognize either the receptor-binding domain of the S1 subunit or S2 subunit of S protein (Table S2), the molecular size of which corresponds to the western blot bands detected in our results. Interestingly, one antibody (GTX632604) band intensity in western blots showed a statistically significantly positive correlation with the anti-S antibody values in serum (Fig. 2). GTX632604 also showed a statistically significant difference in S protein band density in lymph nodes between vaccinated and control cases (Fig. S2). This indicates that higher expressions of S protein in lymph nodes may correlate with higher antibody productions. It also indicates the specificity and reliability of the western blot bands in our study. However, other 4 antibodies failed to show a statistically significant correlation between western blot band intensities and serum anti-S protein values (Fig. 2 and S2). These results indicate limitations of the specificity of the anti-S antibodies. There might be a possibility that the commercially available anti-S antibodies broadly cross-reacts to other human cell components [17]. On the other hand, there is a possibility that the non-infected control cases (both negative for nasal swab S antigen, SARS-CoV-2 N gene PCR, and for serum anti-S and anti-N antibodies) had in fact experienced insidious SARS-CoV-2 infection in the past, then anti-S and anti-N antibody titers had subsided, but the viral S protein RNA had been reverse-transcribed and incorporated into human genome, thus keep producing S protein [18].

Immunohistochemistry of Axillary Lymph Nodes for S Protein

Similar to western blot results, immunohistochemistry of axillary lymph nodes showed S protein positivity both in vaccinated, and control cases (Fig. S1), although GTX635692 showed positive immunohistochemistry in only one vaccinated case. There was no statistically significant difference in numbers of positive cells per square mm in lymph nodes between vaccinated and control cases (Fig. S3). Therefore, discussions similar to western blots can be applied to immunohistochemical results regarding the presence of S protein both in vaccinated cases as well as in control cases. The S protein band intensity in western blots from lymph nodes had no statistically significant correlation with the numbers of anti-S positive immune cells in lymph nodes (Fig. 3).

Conclusion

Five anti-S antibodies detect S protein immunoreactivity by western blots and immunohistochemistry in proteins extracted from human axillary lymph nodes in autopsy specimens. The immunoreactivity was detected in both vaccinated and control (negative for anti-S and anti-N protein antibodies with no known history of vaccination) cases. One anti-S antibody (GTX632604) titers had a statistically significant correlation with S protein immunoreactivity. This S protein immunoreactivity might be due to cross-immunoreactivity due to the possible existence of S proteins from previous corona virus infection other than SARS-CoV-2. There may also a possibility that commercially available anti-S antibodies may react to
other human component proteins. Our results warrant further study on the specificity of commercially available anti-S protein antibodies.

Limitations: Since this is an autopsy tissue study, the functional aspects of immune cells in the lymphocytes are not covered.

**Abbreviations**

SARS-CoV-2  
SARS coronavirus-2  
COVID-19  
coronavirus disease of 2019  
S protein  
Spike protein  
ACE2  
angiotensin converting enzyme 2

**Declarations**

Ethics approval and consent to participate:

The study was approved by Teikyo University Ethical Review Board for Medical and Health Research Involving Human Subjects (Approve No.21-118). Pursuant to the chapter 4 of the ethical guidelines for medical health and health research involving human subjects by the Ministry of Health, Labor and Welfare, individual informed consent was not required. The requirement for informed consent was waived by Teikyo University Ethical Review Board for Medical and Health Research Involving Human Subjects because of the retrospective nature of the study.

All experiments were performed in accordance with the Declaration of Helsinki.

Consent for publication: Not applied.

Availability of data and materials:

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests:

There are no competing interests.

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Authors’ contributions:

MN: Conceptualization, investigation, methodology, writing-original draft, writing-review and editing. HT: investigation, YT: investigation, TA: investigation, TN: conceptualization, investigation, methodology, writing-review and editing. All authors reviewed the manuscript.

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References


**Figures**
Figure 1

Western blots of proteins extracted from lymph nodes.

Antibodies used in the study are shown above each blot.

Case numbers in Table S1 are shown at bottom. For ab272504, MAB10890, and GTX635692, lane gaps between 3 and 4, 4 and 5, and 5 and 6 are abridged. Size markers are at the right or left end.
Figure 2

Correlation between serum anti-S antibody values and signal intensity of S protein bands in western blots from lymph node proteins.

The western blot signal intensity is calculated as the S protein signal intensity divided by the GAPDH signal intensity.

Antibodies were measured by Elecsys Anti-SARS-CoV-2 RUO system.

A statistically significant linear correlation was shown in GTX632604.
Figure 3

Lack of correlation between S protein positive cell numbers and signal intensity of S protein bands in western blots from lymph nodes.

Cell numbers per square millimeter in lymph nodes are compared with signal intensity of S protein bands in western blots from lymph node protein.
Antibodies were measured by Elecsys Anti-SARS-CoV-2 RUO system.

No statistically significant correlation was observed.

Original immunohistochemistry from representative lymph nodes is shown in Supplementary files.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

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- FigS2.tif
- FigS3.tif
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