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Detection of PEG-specific antibodies in SARS-CoV-2 positive and negative sera with implications for autoimmune reactivity

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Polyethylene glycol (PEG) is a stabilizing polymer that may act as an immunogen, as some individuals produce antibodies against it and its covalent attachment to active proteins. We wanted to determine if different titers of PEG antibodies are detected in sera from subjects before and after the SARS-CoV-2 pandemic peak, and if these antibodies possibly contribute to autoimmunity. Using ELISA, we measured IgG, IgM, IgA and IgE antibodies against different sizes of PEG, Moderna COVID-19 vaccine ingredients, and phosphatidylcholine in sera from 90 healthy adults purchased in 2018 that were negative for SARS-CoV-2 IgG antibody, and sera from 90 blood donors purchased in 2023 (collected mid-August 2023) that tested positive for SARS-CoV-2 IgG. To find possible reactivity with human tissue, we also applied both polyclonal and monoclonal antibodies raised in mouse/rabbit specifically against PEG-5K to 65 different human tissue antigens. Analysis of data showed that at 3SD above the mean of the negative control, significant elevations in IgG and IgM antibodies were detected against different sizes of PEG, vaccine ingredients, and phosphatidylcholine. For IgE, significant elevations were found in the PEG-specific antibodies. For IgA antibodies, significant elevation was found only against phosphatidylcholine. The application of monoclonal and polyclonal antibodies made against PEG to 65 different human tissue antigens showed reactions with 20 out of 65 different antigens for rabbit polyclonal, 7 out of 65 for rabbit monoclonal, and 6 out of 65 for mouse monoclonal anti-PEG antibody. Administration of PEGylated products may result in the production of PEG-specific antibodies that may contribute to autoimmunity.

Keywords Polyethylene glycol, SARS-CoV-2, Vaccine, Cross-reactivity, Autoimmunity, Moderna vaccine

Polyethylene glycol (PEG) is a polymer of different sizes that is used for the coating of solid lipid nanoparticles, and has a broad range of applications in manufacturing vaccines and many other products such as pharmaceuticals, cosmetics, household cleaners, and processed foods. This polymer is universally used to maintain the stability of PEGylated products and reduce their uptake by the reticuloendothelial system in order to enhance their therapeutic efficacy and improve their safety after inoculation[1,2].

This widespread use of PEG in so many products was based on the belief that this polymer is inert and non-immunogenic, and that therefore neither cellular nor humoral immune responses may occur against it. It was also believed that PEGylation may prolong a drug's half-life in blood and reduce the immunogenicity of some medications[3].

Based on these characteristics of this highly versatile polymer and a chemical structure of $(CH_2CH_2O)n$, nearly 20 different PEGylated systems have been approved by the FDA for use in many products, and more are under consideration for future approval[2,4]. However, the molecular size of the PEG may have an effect on its immunogenicity. In a study published in 2022[5], rats were injected with 20, 40, or 60 kDa PEG once a week for 24 weeks. A higher prevalence of anti-PEG IgM was observed in the PEG groups, as well as molecular-weight-related increase in PEG in plasma, and molecular-weight-related increase in vacuolation in the spleen, lymph nodes, lungs, and ovaries/testes, without an inflammatory response. These findings indicate that subcutaneous and intravenous exposure to high-molecular-weight PEGs produces anti-PEG IgM antibody responses and tissue vacuolation without inflammation.

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Unfortunately, there is a small number of products that use PEG in different molecular sizes, and in these molecular weights PEG does act as an immunogen, resulting in the production of IgG and IgM anti-PEG antibodies. This anti-PEG antibody production was responsible for the loss of efficacy of some medications in nearly half of the patients receiving PEGylated products in several clinical trials, resulting in the trials' termination[6–10].

This same anti-PEG antibody production could be the result of changes in the immunogenicity of PEG after its conjugation with carrier molecules or particles such as phospholipids, proteins, liposomes, and nanoparticles[3]. The binding of PEG to nanoparticles and other molecules could lead to the production of antibodies, which may result in hypersensitivity reactions that could involve the activation of complement cascade and allergic reaction[3,6–10].

In research conducted in 2015, pre-existing IgG and IgM antibodies were investigated in subjects from the general population who had never undergone treatment with PEGylated medications. Using competitive ELISA, they found IgM in 25%, IgG in 18%, and both IgG and IgM in 30% of the tested individuals[8]. A significant number of these specimens contained very low levels of PEG antibodies, with only 7% exhibiting high levels (> 500 µg/ml) of IgG antibodies, and only about 1% showing such elevation in IgM antibodies[8].

In another study with the participation of more than 1,300 subjects, 23–24% of their sera tested positive for IgG or IgM against PEG, with only 4–9% producing highly PEG-specific antibodies[11].

Finally, another study found that a very small percentage of individuals who were vaccinated with Pfizer/BioNTech mRNA COVID-19 vaccine were found to be positive by skin prick tests, indicating hypersensitivity to PEG which could be IgE-mediated[12–14]. Because these individuals had previous histories of allergic reactions to PEG, and then experienced anaphylaxis after vaccination, it was concluded that antibodies formed after their exposure to PEGylated products played a role in their subsequent hypersensitivities[14–18].

In this current study, we sought to investigate the presence of IgG, IgA, IgM and IgE antibodies against three different PEG products, PEGylated mRNA vaccine, and phosphatidylcholine in the sera of individuals negative for SARS-CoV-2, as well as in individuals with elevated IgG antibody to spike plus nucleoproteins. Furthermore, to study the pathogenicity of PEG antibodies, we examined the reactivity of PEG polyclonal and monoclonal antibodies with 65 different human tissue antigens in order to examine the possible contributions of PEG to autoimmunity.

Methods

Human serum samples from 90 healthy subjects were purchased from Innovative Research in 2018. The samples were aliquoted and kept at − 80 °C until use. An additional 200 healthy sera were purchased from the same source which was collected mid-August 2023 and also aliquoted and kept at −80 °C. These samples classified as healthy were collected from blood donors who were negative for HIV and hepatitis-C. Their ages varied from 20 to 70. All 290 sera were to be tested for the presence of IgG antibody against SARS-CoV-2 spike and nucleoprotein using a kit purchased from Zeus Diagnostic (Zeus ELISA SARS-CoV-2 IgG SM9Z7901G, New Jersey, USA). All 90 samples from 2018 tested negative for SARS-CoV-2 IgG antibody, but 137 out of the 200 sera obtained in 2023 tested positive for SARS-CoV-2 IgG. We selected the top 90 out of the 137 sera that had three-fold or higher than the reference range for SARS-CoV-2 IgG antibody provided in the kit. For example, in the Zeus kit, an ELISA index of 0.9 or less is considered negative, and greater than 0.9 is considered positive. We selected 90 samples with ELISA indices of 2.7 or greater. Thus, we had 90 samples from 2018 that were negative for SARS-CoV-2 IgG antibody, and 90 samples from 2023 that were significantly positive for SARS-CoV-2 IgG antibody.

ELISA for measurement of igG, igA, IgM and IgE antibodies against PEG and associated antigens

For coating ELISA plates, 1 mL of lipid nanoparticles containing mRNA, PEG, and phosphatidylcholine (PEGylated Moderna spike vaccine mRNA-1273, containing messenger ribonucleic acid, SM-102, 117 µg of PEG2000-DMG, cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine per dose) was dissolved in 100 mL of 70% ethanol. Ten mg each of PEG 5 kDa (O-(2-aminoethyl) polyethylene glycol 5,000 Catalog # 672130 [Sigma-Aldrich, Visalia, CA]), PEG-BSA 20 kDa (Multi-PEGylated bovine serum albumin (BSA) (20 kDa mPEG) Catalog # PBSA-00 [Life Diagnostics, Inc., Westchester, PA]), and PEG 40 kDa (8-arm polyethylene glycol 40,000 –NH2, hexaglycerol core, HCl salt Catalog # JKA8012 [Millipore Sigma, Milwaukee, WI]) was dissolved in 200 mL of 0.1 M of carbonate buffer pH 9.6. Vaccine content was diluted serially from 1:2 to 1:32, and a dilution of 1:16 in 0.01 M PBS pH 7.4 was found to be optimal for coating.

The optimal dilution of PEG-5 kDa, PEG-20 kDa, PEG-40 kDa, and phosphatidylcholine (Phosphatidylcholine Catalog # P3556-1G [Sigma-Aldrich, St. Louis, MS]) for coating ELISA plates was determined to be 100 µg/mL, 200 µg/mL, 50 µg/mL, and 25 µg/mL, respectively in carbonate buffer. Each ELISA plate was coated with 100 µL per well of these materials and incubated overnight at 4 °C, followed by an additional eight hours at room temperature. After incubation, the plates were washed five times to remove unbound antigens.

To block nonspecific binding, 200 µL of 2% BSA in 0.01 M PBS was added, and incubation was repeated. After repeated washing, 100 µL of different sera dilutions of 1:100 for IgG and IgM, 1:50 for IgA, and 1:2 for IgE determinations were added to each well. The IgG, IgM and IgA plates were incubated for one hour at room temperature (RT), while the IgE plates were incubated for one hour at RT, followed by incubation overnight at 4 °C. After repeated washing, 100 µL of alkaline phosphatase-labeled anti-human IgG (diluted 1:800), anti-IgM (diluted 1:600), anti-IgA (diluted 1:100) and anti-IgE (diluted 1:200) were added to the proper wells and incubated at RT for 1 h.

Following five washes to remove unbound secondary antibodies, the enzymatic reaction was initiated by adding 1 mg/mL of para-nitrophenylphosphate (PNPP) to all wells. The reaction was stopped 30 min later

by adding 50 µL of 5 N NaOH. The color development was measured at 405 nm using an Epoch Microplate Spectrophotometer from Biotek.

All tests were performed in duplicates, and assays were repeated if variations between replicates exceeded 20%. For each plate, in addition to blank wells (to which no serum was added), two wells were coated with ovalbumin at a concentration of 100 µg/mL and used as negative controls. For positive controls, the serum was replaced with 100 µL of a 1:200 dilution of rabbit anti-PEG antibody, followed by the addition of an anti-rabbit secondary antibody and substrate.

Reaction of sera with low or high levels of PEG antibodies with antigens involved in autoimmunity

Five different sera with no or very low levels of PEG IgG and SARS-CoV-2 antibodies and five different sera with high levels of PEG IgG and SARS-CoV-2 antibodies were diluted at 1:100 and applied to ELISA plates coated with nuclear antigen (NA) Catalog # 708750 INOVA, extractable nuclear antigen (ENA) Catalog # 708615 INOVA, double-stranded DNA (dsDNA) Catalog # 708510 INOVA, actin Catalog # 708785 INOVA, mitochondrial M_2 Catalog # 2338370 Trinity Biotech, rheumatoid factor (RF) Catalog # 708690 INOVA, and C1q complement Catalog # 704620 INOVA following the instructions of the respective kits. The ELISA steps were carried out as instructed, and color development was measured as absorbance (Abs) values at 405 nm. Final values were calculated to determine the antibody reactivity. The Abs were compared to the Abs of the calibrators in the kits, generating ELISA indices.

Reaction of PEG polyclonal and monoclonal antibodies with different tissue antigens

We used ELISA plates coated with antigens such as NA, ENA, dsDNA, RF, actin, mitochondrial M_2 antigen, immune complexes, and an additional 58 tissue antigens, as shown in our earlier study[19]. Different tissue antigens were prepared at an optimal concentration of 10 µg/mL in 0.1 M PBS pH 7.4. 100 µL of each antigen was added to a series of ELISA plates.

After incubation, plates were washed three times using an ELISA washer. To block non-specific binding, 200 µL of 2% BSA in PBS was added to each well and incubated at 4 °C for 24 h.

To examine the binding of PEG antibodies to each one of these antigens, 100 µL of rabbit or mouse anti-PEG antibody (rabbit polyclonal antibody Catalog # PA5-32247 prepared by the injection of PEG-conjugation KLH into rabbits [Invitrogen, Waltham, MA], this antibody reacts to the methoxy group of PEG, and was shown to react strongly to PEG-BSA in Western Blot assay; rabbit monoclonal antibody Catalog # Ab289372 prepared by binding PEG to BSA [Abcam, Waltham, MA], this antibody was shown to react strongly with both PEG-3 kDa and PEG-24 kDa conjugated to ovalbumin; mouse monoclonal Anti-PEG Catalog # Ab275332 [Abcam, Waltham, MA], Western blot showed that this antibody reacted to PEG conjugated to KLH [keyhole limpet hemocyanin]) at optimal dilution of 1:200 were added to duplicate wells of microtiter plates coated with the corresponding antigens.

After 1 h of incubation and subsequent washing, alkaline phosphatase-labeled anti-rabbit or anti-mouse IgG antibodies at optimal dilutions were added to the appropriate plates and incubated for 1 h at RT. Plates were washed five times to remove unbound antibodies, and 100 µL of substrate (PNPP) were added to each well. Color development was measured after 30 min using an ELISA reader at 405 nm. The means of Abs values from quadruplicate wells were calculated and used for graphical representation.

The percentage of tissue reaction with each antibody was calculated using the following formula:

$$\%\ \text{of reaction with the antibody} = \frac{\text{Abs of tissue reactivity} - \text{Abs of background*}}{\text{Abs of PEG reactivity} - \text{Abs of background}} \times 100$$

*The background is the BSA mean + 3SD.

To determine the specificity of mouse monoclonal and rabbit polyclonal antibodies binding to different tissue antigens, these antibodies were replaced with the same dilution of human serum from a healthy individual or non-immunized rabbit serum. The replacement sera were added to quadruplicate wells for comparison.

Additionally, as negative controls, four wells coated with 2% human serum albumin (HSA) and four wells coated with 2% BSA alone were included. After adding the other reagents to these control wells, the Abs were measured.

Binding of serially diluted PEG antibodies to tissue antigens

To demonstrate the specificity of PEG antibody reactions with tissue antigens, serially diluted polyclonal anti-PEG antibodies were bound to different strips of ELISA plates, each containing 8 wells. Each strip was coated with PEG, actin, phospholipid, or myelin oligodendrocyte glycoprotein (MOG) antigen at their optimal concentrations. These antigens were specifically chosen since they showed moderate to strong reactions to polyclonal PEG antibody. MOG was chosen because it did show some reaction albeit minimal with PEG antibody. The PEG antibody was then serially diluted from 1:200 to 1:25,600 and added to the ELISA plate wells.

After incubation, washing, and completion of all other steps of the ELISA procedure, the Abs values were measured and recorded.

Inhibition of PEG antibody binding to different antigens in the presence of PEG and other antigens

Inhibition assays were conducted with specific and non-specific antigens to further demonstrate the specificity of PEG antibody binding to human tissue antigens,. This procedure was designed to demonstrate the specificity of the PEG antibody binding to human tissue antigens.

Four different strips of ELISA microtiter plate were coated either with PEG, MOG, phospholipid, or actin. 100 µL of serum diluent were added to the first wells (well #1) of all four strips. Then, from the second wells to the eighth wells (wells #2-#8), antigens in increasing amounts, 2 µg, 4 µg, 8 µg, 16 µg, 32 µg, 64 µg, and 128 µg, were added respectively to each well, with PEG for strip #1, MOG for strip #2, phospholipid for strip #3, and actin for strip #4.

Immediately, 100 µL of a 1:200 dilution of polyclonal anti-PEG antibody made in diluent was added to all wells of each strip. The strips were incubated for 1 h at RT. After incubation, washing, the addition of anti-rabbit IgG labeled with enzyme, and the completion of all ELISA steps, the Abs values were recorded at 405 nm, and the inhibition of anti-PEG antibody binding to different antigens in proportion to the increased concentration of different antigens in liquid phase is shown in Table 2.

Statistical analysis

Statistical analysis was performed by comparing the Abs obtained by applying sera negative for SARS-CoV-2 antibodies versus sera positive for SARS-CoV-2 antibodies to the tested antigens. Statistical analysis was also performed by comparing the Abs obtained from the reaction of PEG antibody with the tested tissue antigens to the mean + 3SD of the background with no antigens. Independent parametric t-tests using Excel were performed to evaluate mean differences of Abs between controls and antigens. A Bonferroni adjustment was conducted to account for type 1 errors with multiple comparisons with a desired overall alpha level of 0.05 and 50 tests, a = 0.05/65 = 0.00076.

Ethics declarations

We purchased mouse monoclonal and rabbit monoclonal and polyclonal antibodies from certified, regulated commercial sources who use immunization protocols for the animals that conform to The Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, publication no 85-23, 1985.

We obtained from commercial sources human sera which were unidentifiable or de-identified, and, as such, under the Common Rule or government regulation 45 CFR 46, this study is not human subjects research since we are using samples for which donor information cannot be readily ascertained. In such cases, IRB review is not required, and the investigator makes and certifies this determination.

Results

IgG, IgA, IgM, and IgE antibodies against PEG-5k, PEG-40k, multi-PEGylated BSA (20 kDa), PEGylated vaccine antigens, and phosphatidylcholine were measured by ELISA in 90 SARS-CoV-2 spike protein and nucleocapsid antibody-negative sera and 90 SARS-CoV-2 spike protein and nucleocapsid antibody-positive sera. Significance as expressed by p values were calculated by comparing the Abs of each antigen's negative controls with the Abs of each antigen's SARS-CoV-2-positive readings. Elevation percentages were calculated based on the mean + 3SD of the controls for each antigen.

For IgG antibody, the differences in Abs between control sera negative for SARS-CoV-2 antibody and sera positive for SARS-CoV-2 antibody were highly significant for all antigens, as shown by their p values ($p < 0.0001$). The elevation percentages for all samples, both those positive and those negative for SARS-CoV-2 antibody, are based on a cutoff of control mean + 3SD. For example, of the 90 samples negative for SARS-CoV-2 antibody, 8% showed a level of PEG 20 K IgG antibodies above the cutoff, which was the mean of the negative controls + 3SD. For the 90 samples positive for SARS-CoV-2 antibody, 37% showed a level of PEG 20 K IgG antibodies above the cutoff, which was the mean of the negative controls + 3SD. The percentages for the other antigens are shown in Fig. 1.

For IgM antibody, the differences in Abs between control sera negative for SARS-CoV-2 antibody and sera positive for SARS-CoV-2 antibody were highly significant for all antigens, as shown by their p values ($p < 0.0001$). The elevation percentages for all samples, both those positive and those negative for SARS-CoV-2 antibody, are based on a cutoff of control mean + 3SD. For example, of the 90 negative samples, 8% showed a level of PEG 20 K IgM antibodies above the cutoff (mean of the negative controls + 3SD). For the 90 positive samples, 44% showed a level of PEG 20 K IgM antibodies above the cutoff. The percentages for the other antigens are shown in Fig. 2.

For IgA antibody, the differences in Abs between negative controls and SARS-CoV-2-positive sera were non-significant for nearly all the antigens, except for phosphatidylcholine with $p = 0.0181$. The elevation percentages for all samples, both those positive and those negative for SARS-CoV-2 antibody, are based on a cutoff of control mean + 3SD. For example, of the 90 negative samples, 3% showed a level of PEG 20 K IgA antibodies above the cutoff (mean of the negative controls + 3SD). For the 90 positive samples, 7% showed a level of PEG 20 K IgA antibodies above the cutoff. The percentages for the other antigens are shown in Fig. 3.

For IgE antibody, the differences in Abs between controls and SARS-CoV-2-positive sera were significant for all the PEG products, while vaccine components and phosphatidylcholine were both non-significant respectively with $p = 0.4534$ and $p = 0.0552$. The elevation percentages for all samples, both those positive and those negative for SARS-CoV-2 antibody, are based on a cutoff of control mean + 3SD. For example, of the 90 negative samples, 7% showed a level of PEG 20 K IgE antibodies above the cutoff. For the 90 positive samples, 20% showed a level of PEG 20 K IgE antibodies above the cutoff. The percentages for the other antigens are shown in Fig. 4.

Reaction of human sera with very low or very high levels of PEG antibodies with tissue antigens

Using different ELISA plates coated with antigens associated with autoimmunity, five different sera with low levels of PEG-specific IgG antibodies (mean ELISA index: 0.9 ± 0.35) and five different sera with very high levels of PEG-specific IgG antibodies (mean ELISA index: 2.7 ± 0.9) were examined for their degree of reactivity with these antigens.

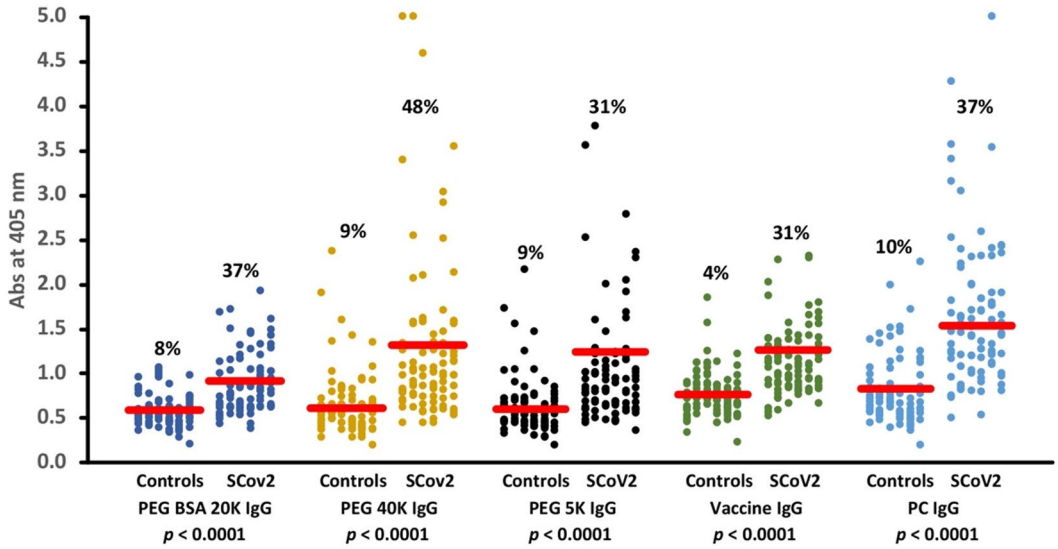

Fig. 1. Detection of IgG antibodies against different PEGs, vaccine components and phosphatidylcholine in controls negative for SARS-CoV-2 vs. samples positive for SARS-CoV-2. The means for negative controls and SARS-CoV-2 positives are shown by the red bars. Elevation percentages were calculated based on the mean + 3SD of the controls for each antigen. The differences between the levels of IgG antibodies in the sera of controls versus SARS-CoV-2-positive sera are highly significant for all five tested antigens, as shown by the *p* values ($p < 0.0001$), which were derived from parametric Excel t-testing. All differences in Abs were highly significant and are shown in the graph. SCoV2 = SARS-CoV-2-positive. PEG, polyethylene glycol; PC, phosphatidylcholine. $n = 90$.

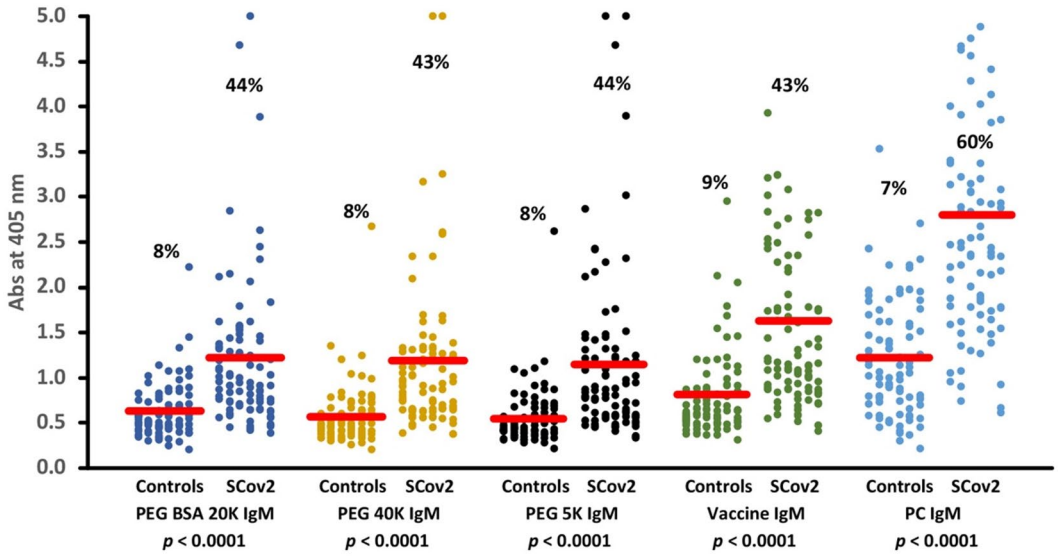

Fig. 2. Detection of IgM antibodies against different PEGs, vaccine components and phosphatidylcholine in controls negative for SARS-CoV-2 vs. samples positive for SARS-CoV-2. The means for negative controls and SARS-CoV-2 positives are shown by the red bars. Elevation percentages were calculated based both on the mean + 3SD of the controls for each antigen. The differences between the levels of IgM antibodies in the sera of controls versus SARS-CoV-2-positive sera are highly significant for all five tested antigens, as shown by the *p* values ($p < 0.0001$), which were derived from parametric Excel t-testing. All differences in Abs were highly significant and are shown in the graph. SCoV2 = SARS-CoV-2-positive. PEG, polyethylene glycol; PC, phosphatidylcholine. $n = 90$.

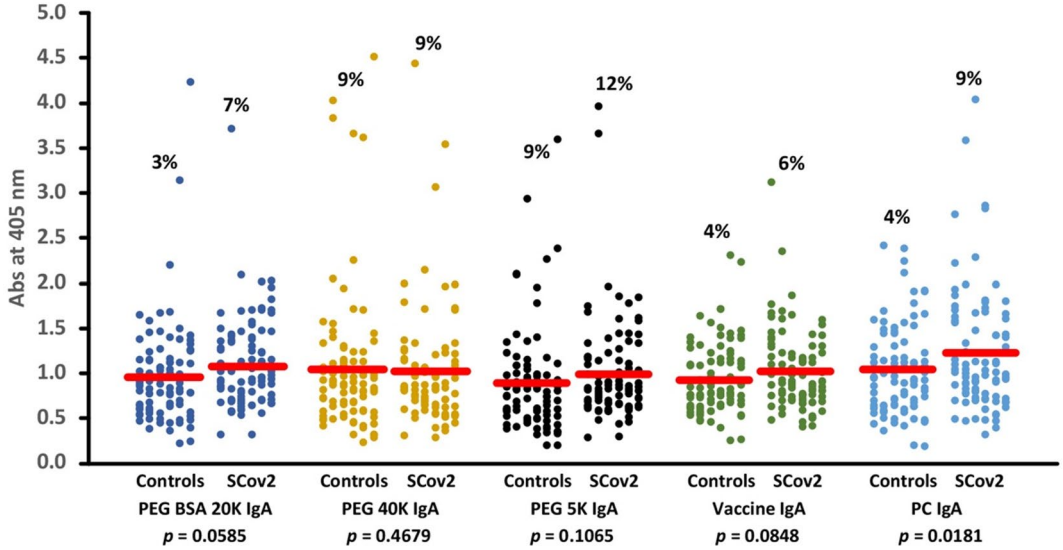

Fig. 3. Detection of IgA antibodies against different PEGs, vaccine components and phosphatidylcholine in controls negative for SARS-CoV-2 vs. samples positive for SARS-CoV-2. The means for negative controls and SARS-CoV-2 positives are shown by the red bars. Elevation percentages were calculated based both on the mean + 3SD of the controls for each antigen. The differences between the levels of IgA antibodies in the sera of controls versus SARS-CoV-2-positive sera are non-significant for nearly all tested antigens, except for phosphatidylcholine with p value = 0.0181. p values were derived from parametric Excel t-testing. SCoV2 = SARS-CoV-2-positive. PEG = polyethylene glycol. PC = phosphatidylcholine. $n = 90$.

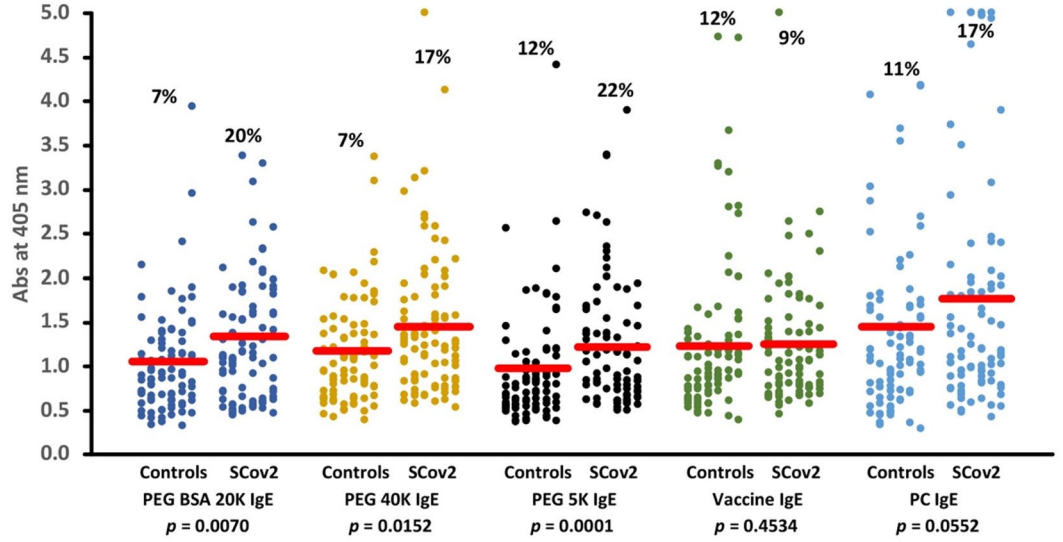

Fig. 4. Detection of IgE antibodies against different PEGs, vaccine components and phosphatidylcholine in controls vs. patients positive for SARS-CoV-2. The means for negative controls and SARS-CoV-2 positives are shown by the red bars. Elevation percentages were calculated based both on the mean + 3SD of the controls for each antigen. The differences between the levels of IgE antibodies in the sera of controls versus SARS-CoV-2-positive sera are significant for all PEG products, while vaccine components and phosphatidylcholine are non-significant. p values were derived from parametric Excel t-testing. SCoV2, SARS-CoV-2-positive; PEG, polyethylene glycol; PC, phosphatidylcholine. $n = 90$.

Data presented in Fig. 5 show that the reactivity of PEG-positive sera with actin was significantly higher than that of sera with low positive PEG antibody levels. For actin, three of the five samples (27.1, 29.3, 34.2) had higher reactivity than the cutoff of < 20 from the kit, resulting in a very significant p value of 0.0095. with all samples higher than the kit cutoff of < 4.4. C1q was also significant with $p = 0.0495$, while the rest of the antigens had non-significant reactions, with dsDNA at $p = 0.1287$, rheumatoid factor at $p = 0.1804$, ENA at $p = 0.2342$, and mitochondrial at $p = 0.2611$, and C1q almost significant with a p value of 0.05.

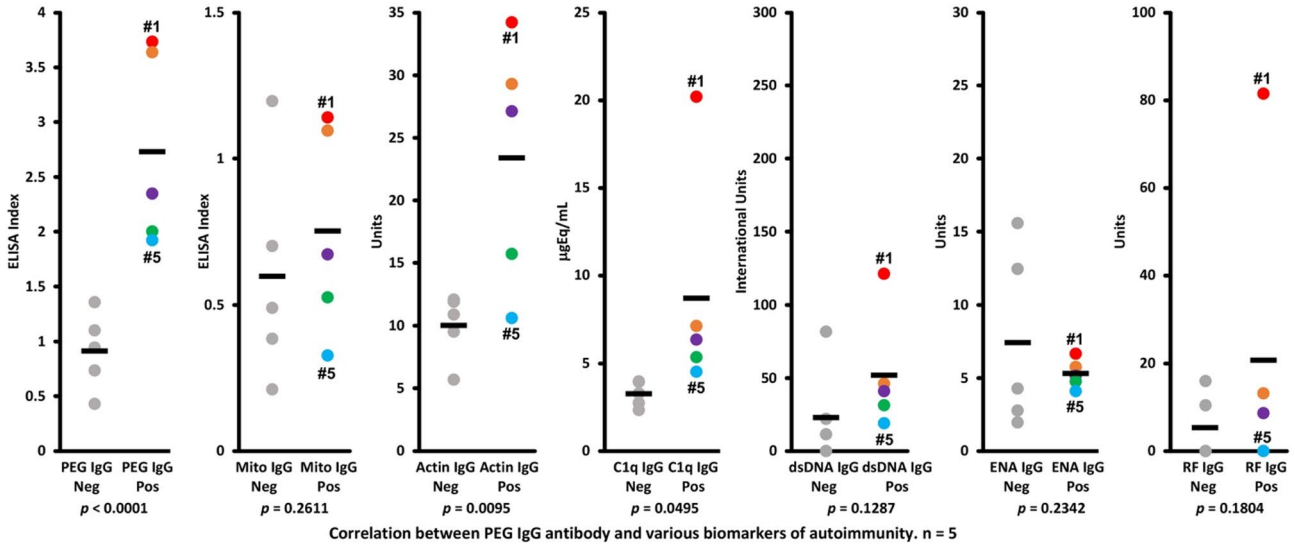

Fig. 5. Reaction of sera negative and positive for PEG IgG antibody with various antigens used as biomarkers of autoimmunity. The reaction of PEG was most significant with actin ($p = 0.009$). C1q was also significant with $p = 0.0495$. The reactions were non-significant mitochondrial, dsDNA, ENA and RF. p values were derived from parametric Excel t-testing. $n = 5$.

While the reactivity of PEG-positive sera with RF yielded a value (8.7 ± 6.5) significantly greater than the cutoff provided in the kit (< 6.0) (Fig. 5), the results for both mitochondrial and dsDNA antigens were within the cutoffs of their ELISA kits.

For ENA-coated plates, it is interesting to note that the reaction of PEG-negative sera yielded higher EU values (11.2 ± 6.2) than PEG-positive sera (5.3 ± 0.9), but both values were below the cutoff of < 20 EU for ENA. This dot plot (Fig. 5) analysis further revealed that sample #1, which had the highest PEG antibody level (marked in red), also showed the highest reactivity with all six tested antigens, whereas sample #5 (marked in blue) with a relatively much lower PEG IgG level showed the lowest reactivity with all tested antigens.

Reaction of monoclonal and polyclonal anti-PEG antibodies with human tissue antigens

We measured the reactivity of mouse monoclonal, rabbit monoclonal, and rabbit polyclonal anti-PEG antibodies with 65 different tissue antigens. Unimmunized mouse and rabbit sera served as controls and showed no significant reactivity with any of the 65 tissue antigens (Abs values were similar to the ELISA background, < 0.18).

When anti-PEG antibodies were applied to ELISA plates coated with PEG and the 65 tissue antigens, all three tested antibodies reacted very strongly with the PEG-coated ELISA plate wells with Abs of 3.6, 3.7 and 3.8, which are close to the maximum ELISA detection limit of 4.0. We considered this reaction between PEG antibody and PEG as 100% (see Figs. 6, 7 and 8).

1. Mouse monoclonal anti-PEG antibodies reacted significantly with myelin basic protein (MBP), MOG, NA, ENA, actin, and dsDNA (reactivity ranged from 11 to 42%). Reactivity with other 59 antigens resulted in Abs values of < 0.2, close to the background level, and is non-significant (Fig. 6).

2. Rabbit monoclonal anti-PEG antibodies showed weaker reactivity with tissue antigens compared to mouse monoclonal antibodies. For example, reactivity with NA and neurofilaments ranged from 13.2 to 13.4%, and reactivity with insulin, myoglobin, histone, islet cell antigen, and phosphatidylcholine ranged from 8.4 to 12.4%. No significant reaction was observed with the remaining 58 antigens (Fig. 7).

3. Rabbit polyclonal anti-PEG antibodies had low to very high reactions (10–68%) with 20 out of 65 tissue antigens. These 20 antigens came from diverse groups that included brain, heart, clotting factors, thyroid, muscle, skin, mitochondria, and antigens used for autoimmune screening. Using 0.3 Abs as the cutoff point, we observed that the strongest reactions were with phospholipid (Abs 2.6), actin (Abs 1.7), myelin-associated glycoprotein (MAG) (Abs 1.3), TPO (Abs 1.3), myosin (Abs 1.0), mitochondrial antigen (Abs 0.9), phosphatidylcholine (Abs 0.8), and MBP (Abs 0.76), while the other antigens had Abs ranging from 0.33 to 0.67 (Fig. 8). The reactivity of PEG-specific antibodies with various tissue antigens, including calculated Abs values and calculated % reactivity, is summarized in Table 1. The Abs for osteocytes, aquaporin and islet cell antigen were all borderline.

Demonstration of PEG antibody specific binding to tissue antigens

The specificity of PEG polyclonal antibody binding to human tissue was confirmed through serial dilution and inhibition studies. As shown in Fig. 9, binding of PEG antibodies to PEG-coated or cross-reactive antigen-coated ELISA wells declined in proportion to antibody dilution from 1:200 to 1:25,600. For example, the reaction of

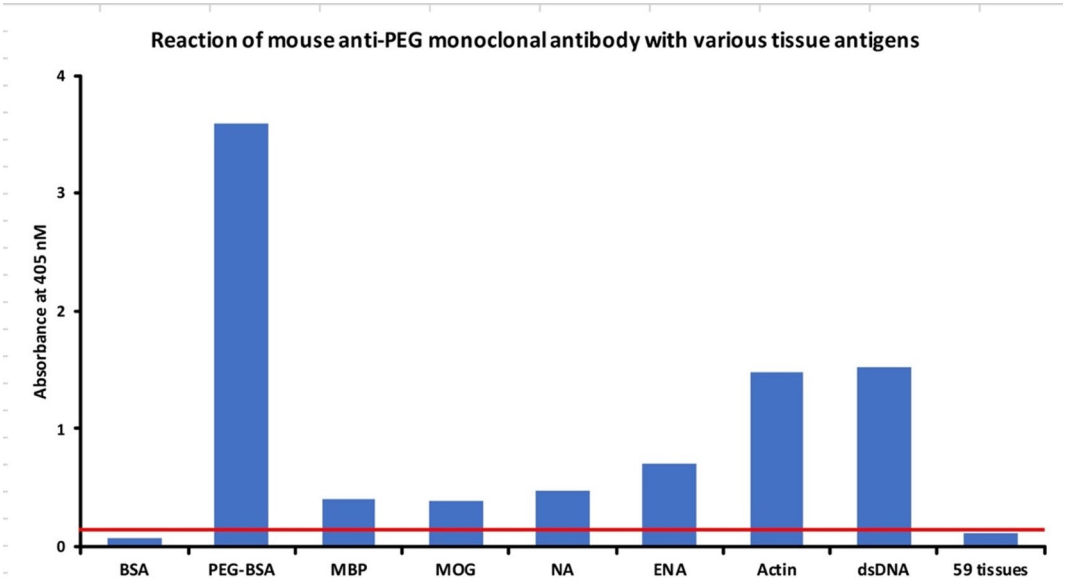

Fig. 6. Reaction of mouse anti-PEG monoclonal antibody with various tissue antigens. The red line indicates the background mean + 3SD. Anything above the red line is significant. Anything below the red line is non-significant. PEG-BSA with 3.6 Abs is considered 100%, MBP, MOG, NA, ENA, actin, dsDNA are considered significant with 11–42%, and the other 59 tissues are non-significant.

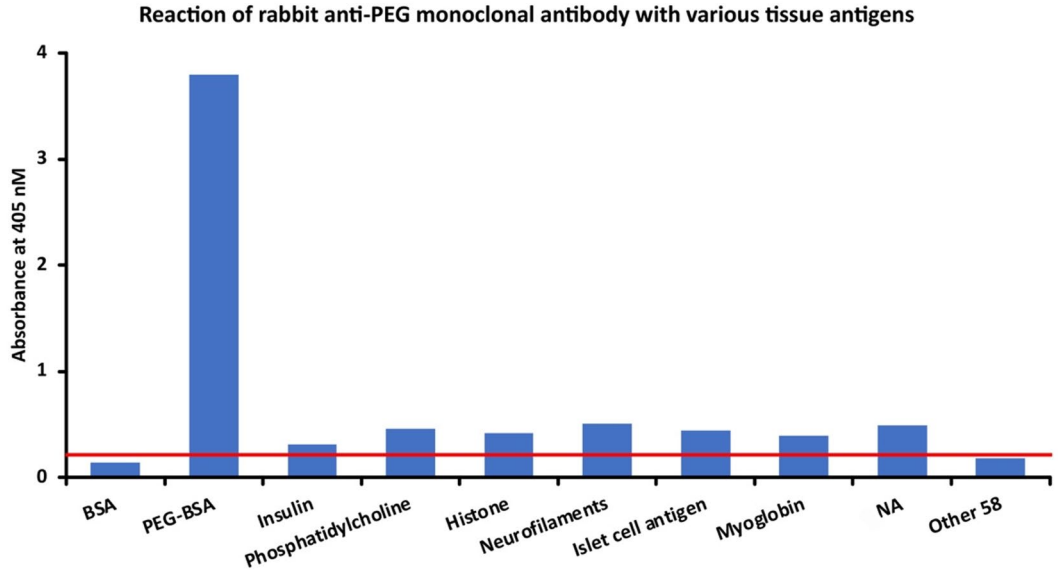

Fig. 7. Reaction of rabbit anti-PEG monoclonal antibody with various tissue antigens. The red line indicates the background mean + 3SD. Anything above the red line is significant. Anything below the red line is non-significant. PEG-BSA with 3.7 Abs is considered 100%, insulin, phosphatidylcholine, histone, neurofilaments, islet cell antigen, myoglobin and nuclear antigen (NA) are significant with 8–13%, and the other 58 tissues are non-significant.

anti-PEG antibody with PEG at a dilution of 1:200 gave an Abs of 3.76, while the dilution of 1:25,600 resulted in an Abs of 0.82. Likewise, reaction with phospholipid at 1:200 dilution was 2.38, but declined at 1:25,600 dilution to 0.47 (Fig. 9). Similar results were obtained when serially diluted PEG polyclonal antibody was applied to actin and MOG. To further demonstrate the specificity of these antibody reactions, an inhibition study was performed by the addition of PEG, phospholipid, actin and MOG in concentrations ranging from 0 to 128 μg in the liquid phase of the ELISA process. Compared to the baseline uninhibited reaction of PEG antibody with PEG or cross-reactive antigens, the addition of increased concentrations of PEG or cross-reactive antigens resulted in significant inhibition of PEG antibody binding to PEG and to cross-reactive antigens in proportion to the degree

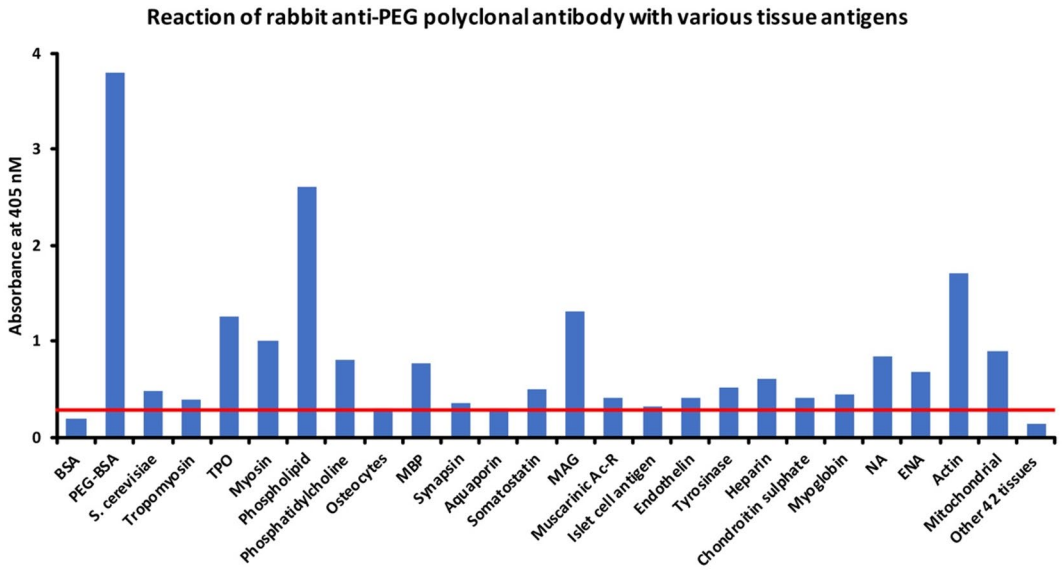

Fig. 8. Reaction of rabbit anti-PEG polyclonal antibody with various tissue antigens. The red line indicates the background mean + 3SD. Anything above the red line is significant. Anything below the red line is non-significant. PEG-BSA with 3.8 Abs is considered 100%, phospholipid (68%), actin (45%), myelin-associated glycoprotein (MAG) (34%) and thyroid peroxidase (TPO) (33%) are the most significant, other antigens are positive ranging from 7.9 to 26.3%. At + 3SD above the mean, three tissues, osteocyte, aquaporin and islet cell antigen are borderline, while the other 42 tissues are non-significant.

of antibody reactivity. For example, the inhibition in the binding of anti-PEG to phospholipid and actin was pronounced in the presence of phospholipid and actin respectively (Table 2).

Discussion

In this study, we sought to characterize the levels of IgG, IgA, IgM, and IgE isotype antibodies against PEG in the sera of blood donors, from both those which tested negative and those which tested positive for SARS-CoV-2 antibodies. These antibodies were measured against PEG size 5 kDa, PEG 40 kDa, multiple PEGylated BSA 20 kDa and the SARS-CoV-2 mRNA vaccine, which contains minute amounts of mRNA but relatively significant amounts of PEG, phosphatidylcholine, and nanoparticles. Thus, we included the entire contents of the vaccine as antigens in our antibody assays.

The BNT162b2 (Pfizer-BioNTech) vaccine contains 50 ALC-0159 per dose, while the Moderna spike vaccine (mRNA-1273) used in this study contains 117 µg of PEG2000-DMG per dose[20]. Both ALC-0159 and PEG2000-DMG contain methoxy groups, which increase their immunogenicity[21,22].

Due to the widespread use of PEGylated products in daily life and the immunogenicity of different PEGs, particularly those with methoxy groups, anti-PEG antibodies have been detected in approximately 25% of blood donors and in up to 42% of patients without any history of treatment with known PEGylated pharmaceuticals[23,24]. A study by Yang Q et al.[25] found that anti-PEG antibodies were detected in around 56% of serum samples collected from 1970 to 1999 (IgG 20%, IgM 19%, both IgG and IgM 16%), while anti-PEG antibodies were found in around 72% of contemporary samples (IgG 18%, IgM 25%, both IgG and IgM 30%). In different studies the prevalence rate of pre-existing anti-PEG was reported as low as 1% and as high as 44%[8,26,27]. In more recent studies involving healthy donors, or treatment-naïve patients, this percentage ranged from 20 to 30%[7,11,28,29].

Based on their own findings, Yang et al.[25] did not accept the hypothesis of emerging anti-PEG prevalence, and concluded instead that immunological responses to PEG are likely longstanding due to increased parenteral use of PEGylated products in clinical, pharmaceutical, food, beauty, and healthcare industries. Our own data regarding percentage elevation in IgG, IgM and IgE antibodies measured against different PEGs is in agreement with the immunological theory proposed by Yang et al.[25].

Overall, our findings regarding the prevalence rates for the so-called pre-existing IgG and IgM anti-PEG antibodies of 0–8% differ substantially from the previously reported rates of 20–30% for healthy donors or treatment-naïve patients, which would correspond to our SARS-CoV-2 negative antibody group[7,11,28,29]. This low prevalence of IgG and IgM against all three PEGs used in our study is likely attributable in part to the PEGs used as antigens in our assay or perhaps to our use of higher threshold values.

Whereas the rates for the three different PEGs are fairly similar for IgG, IgA, IgM and IgE antibodies for sera negative for SARS-CoV-2, the sera positive for SARS-CoV-2 show significant differences between IgG and IgM in comparison to IgA and IgE. The elevation percentages for the positive IgG and IgM for all three PEGs are roughly three to six-fold of their negative counterparts, while the IgA and IgE positive readings run from almost the same as the negative readings to barely double.

Antigen	Rabbit polyclonal (Abs)	% Reactivity	Rabbit monoclonal (Abs)	% Reactivity	Mouse monoclonal (Abs)	% Reactivity
PEG-BSA	3.8	100 ++++	3.7	100 ++++	3.6	100 ++++
Nuclear antigen	0.84	22 +	0.49	13.2 +	0.48	13.3 +
Extractable nuclear antigen	0.67	17.6 +	NS	NS	0.71	19.7 +
Double-stranded DNA	NS	NS	NS	NS	1.52	42.2 ++
Actin	1.7	44.7 +++	NS	NS	1.48	41.1 ++
Histone	NS	NS	0.42	11.3 +	NS	NS
Mitochondrial (M2)	0.90	23.7 +	NS	NS	NS	NS
Myoglobin	0.45	11.8 +	0.39	10.3 +	NS	NS
Saccharomyces cerevisiae	0.48	12.6 +	NS	NS	NS	NS
Tropomyosin	0.39	10.2 +	NS	NS	NS	NS
Myosin	1.0	26.3 ++	NS	NS	NS	NS
Thyroid peroxidase	1.25	32.9 ++	NS	NS	NS	NS
Phospholipid	2.6	68.4 ++++	NS	NS	NS	NS
Phosphatidylcholine	0.8	21 +	0.46	12.4 +	NS	NS
Osteocyte	0.3	7.9 +	NS	NS	NS	NS
Somatostatin	0.5	13.1 +	NS	NS	NS	NS
Myelin basic protein	0.76	20 +	NS	NS	0.40	11.1 +
Myelin oligodendrocyte glycoprotein	0.15	3.9	NS	NS	0.39	10.8 +
Myelin-associated glycoprotein	1.3	34.2 ++	NS	NS	NS	NS
Neurofilament protein	0.16	4.2	0.51	13.4 +	NS	NS
Aquaporin	0.3	7.9 +	NS	NS	NS	NS
Synapsin	0.35	9.2 +	NS	NS	NS	NS
Heparin	0.6	15.8 +	NS	NS	NS	NS
Chondroitin sulfate	0.4	10.5 +	NS	NS	NS	NS
Muscarinic acetylcholine receptor	0.41	10.8 +	NS	NS	NS	NS
Endothelin	0.41	10.8 +	NS	NS	NS	NS
Islet cell antigen	0.31	8.1 +	0.44	11.9 +	NS	NS
Insulin	NS	NS	0.31	8.4 +	NS	NS

Table 1. % Reactivity of rabbit polyclonal, and rabbit and mouse monoclonal antibodies against PEG with PEG and different tissue antigens. 5–25% = +; 25.1–40% = ++; 40.1–55% = +++; >55% = ++++. NS indicates "Non-significant" or values below detection limits. Data are presented as percentages of reactivity for each antibody with the given antigen. "PEG-BSA" refers to polyethylene glycol conjugated with bovine serum albumin.

These results support findings in animal models, where anti-PEG antibodies were detected against PEG backbone repeats and PEG terminal groups such as methoxy and hydroxy moieties[22,30]. The PEG backbone seems to be common to all PEGylated products, whereas the terminal groups can vary from product to product[7,28]. The specificity of anti-PEG antibodies has been demonstrated in different studies[7,26] and in this study using PEG in competition assay, as well as by serial dilutions (Fig. 9).

The very high prevalence of IgG and IgM antibodies in SARS-CoV-2 IgG positive individuals in comparison to the negative donors (as shown in Figs. 1 and 2) could be attributed to the introduction of the successful mRNA anti-COVID-19 vaccines containing PEG for the stabilization of lipid nanoparticles[31]. From the 200 samples we had obtained in 2023, we had selected 90 different blood samples for the study based on a SARS-CoV-2 spike and nucleoprotein IgG antibody level of 2.7 or greater, which is three-fold higher than the reference range provided by the kit manufacturer. Although we lacked accurate vaccination history for the selected 90 study participant samples, our data suggests the possibility that such a rise in IgG antibody against SARS-CoV-2 is due to repeated vaccination with the mRNA vaccine containing PEGylated stabilizers.

There is other support for this, as in a very elegant study[32], Ju et al. studied plasma from 130 adults for PEG-specific antibodies. The 130 were composed of three separate groups: 55 unvaccinated subjects (40 convalescent SARS-Cov-2 infected patients and 15 healthy donors), 55 adults who received two doses of BNT162b2 vaccines, and 20 adults who received two doses of mRNA-1273 vaccines. Anti-PEG IgG and IgM antibodies commonly detected in a small percentage prior to vaccination were significantly boosted after the second vaccination by a mean of 13.1-fold for IgG and 68.5-fold for IgM antibodies when mRNA-1273 was used. This elevation in IgG and IgM antibodies ranged from only 1.78 to 2.64-fold following vaccination with BNT162b2[32]. Interestingly, the rise in PEG-specific antibodies was associated with the systemic reactogenicity and increase in phagocytosis of PEG particles in human blood[32].

The investigators of this study[32] used titers for their measurements, and for the two cohorts that received vaccinations, using an end point titer of > 1:10, they found that even prior to the vaccinations, PEG-specific IgG was detectable in 53 (71%) of the combined 75 subjects (55 + 20) ranging in titer from 1:12 to 1:3000. For our

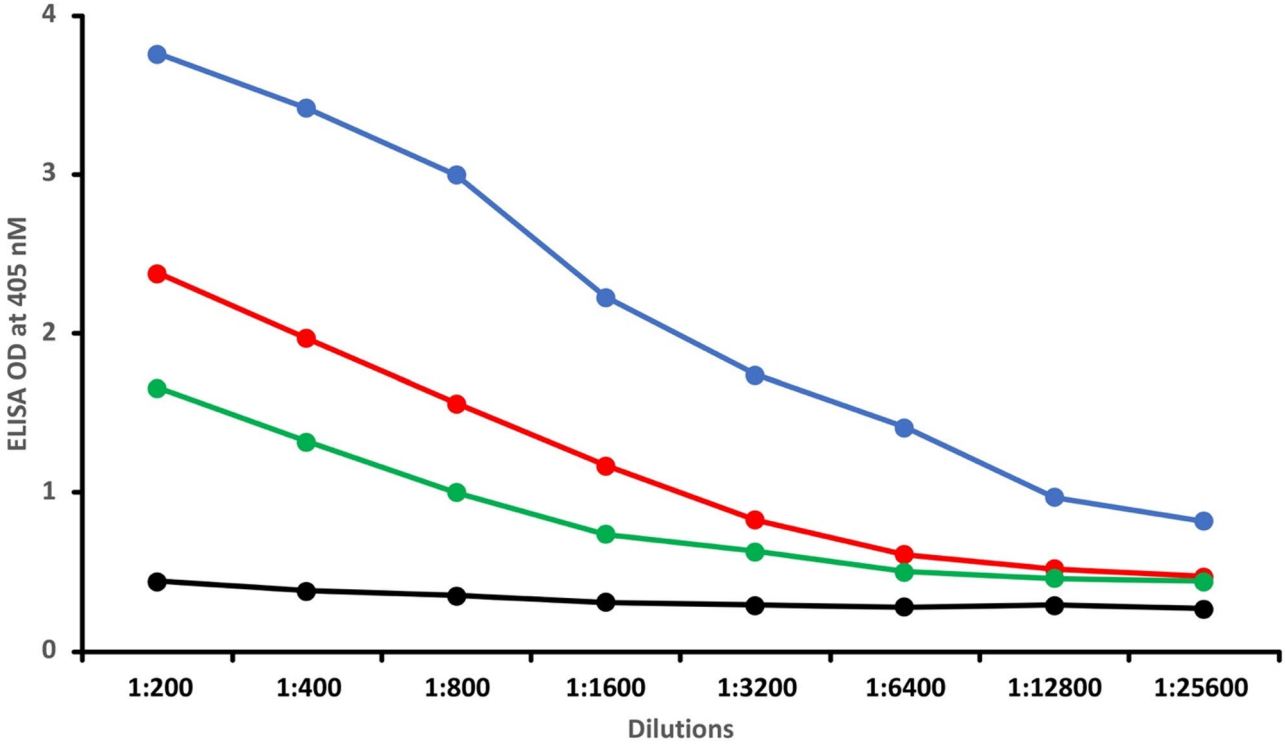

Fig. 9. Dilution of rabbit polyclonal anti-PEG antibody and its reaction with PEG and other antigens. ● PEG with Polyethylene glycol (PEG). ● PEG with Phospholipid. ● PEG with Actin. ● PEG with Myelin oligodendrocyte glycoprotein (MOG).

	Inhibition with							
	0	2	4	8	16	32	64	128
1. Anti-PEG reaction with PEG in the presence of 0–128 µg of PEG	3.76	3.42	3.0	2.23	1.74	1.41	0.97	0.82
2. Anti-PEG reaction with Phospholipid in the presence of 0–128 µg of Phospholipid	2.38	1.97	1.56	1.17	0.83	0.61	0.52	0.47
3. Anti-PEG reaction with Actin in the presence of 0–128 µg of Actin	1.66	1.32	1.0	0.74	0.63	0.50	0.46	0.44
4. Anti-PEG reaction with MOG in the presence of 0–128 µg of MOG	0.44	0.38	0.35	0.31	0.29	0.28	0.29	0.27

Table 2. Inhibition of PEG antibody binding to PEG and cross-reactive antigens.

own study, using the mean Abs values as cutoff points for the SARS-CoV-2 IgG negative samples, we found that 8–9% of the sera showed elevation in pre-existing PEG antibodies. Our measurement methodologies may differ from each other, but there is a commonality in that both studies found elevations in supposedly unvaccinated individuals.

In a 2023 study[33], PEG-specific IgG antibody levels increased from a baseline of 36.7–58.2%, while IgM antibody levels rose from 13.9 to 39.2% three weeks after the first dose of a vaccine (Comirnaty® (mRNA-LNP-based SARS-CoV-2 vaccine). Notably, no anti-PEG IgE antibodies were detected in either vaccinated or unvaccinated donors[33]. This lack of detected PEG-specific IgE antibodies in donors before and after vaccination is in contrast with our findings of detected IgE anti-PEG in 7–12% of the controls (negative SARS-CoV-2 IgG) and its increase to 17–22% in sera highly positive for SARS-CoV-2 IgG against three different PEG products (see Fig. 4). According to Bavli Y et al.[33], the reason for this discrepancy in the detection of PEG-specific IgE antibody may stem from the dilution of sera by a minimum of 1:25. In most clinical and research laboratories, for the detection of IgE, serum is either added directly to the antigen or diluted at a maximum of 1:2 in serum diluent, and not 1:25. Furthermore, according to that study's authors, they used peroxidase-labeled secondary antibody which was read at 405 nm. Peroxidase-labeled secondary antibody is supposed to be read at 450 nm. The wrong dilution factor and wrong nm reading setting for IgE leads us to question the accuracy of this experiment's finding regarding their detection of IgE antibodies.

In our own study, we detected IgE-specific anti-PEG antibodies at low levels for all three PEG products in samples negative for SARS-CoV-2 antibody; 7% for PEG BSA 20 K, 7% for PEG 40 K, and 12% for PEG 5 K. This percentage for the PEG products was increased in sera highly positive for SARS-CoV-2 to 17–22%, or by 1.8- to 2.8-fold. The p values for all three PEG products were significant (PEG BSA 20 K $p = 0.0070$; PEG 40 K $p = 0.0152$; PEG 5 K $p = 0.0001$). The differences in antibody elevations between controls and positive samples for both

vaccine components and phosphatidylcholine were not significant, with $p = 0.4534$ and $p = 0.0552$ respectively (see Fig. 4).

Elevation percentages for IgA anti-phosphatidylcholine were 4% for the SARS-CoV-2 negative group and 9% for the positive group. Phosphatidylcholine was the only one of the five antigens in the IgA group to have a significant difference in antibody elevation between controls and SARS-CoV-2-positive sera with $p = 0.0181$ (see Fig. 3).

These findings may indicate that antibodies against phosphatidylcholine may contribute to the detection of the so-called pre-existing antibodies and their enhancement in humans following vaccination using lipid nanoparticles, possibly due to cross-reactivity[34,35].

A diverse range of natural mixtures of cholesterol, phospholipids, sphingolipids, and PEGylated lipids are involved in the production of lipid nanoparticles which are used to deliver protein, nucleic acids and other factors to their biological targets[36,37]. However, the antigenicity of these lipids and phospholipids may trigger immune responses in the form of antibodies against them, leading to adverse reactions and immune activation.

We have already shown how the measurements of IgG and IgM antibodies against the five tested antigens, including vaccine and phosphatidylcholine, were much more significant than those for IgA and IgE. These significant elevations in IgG and IgM antibodies against a broad range of PEGs and vaccine contents in sera positive for SARS-CoV-2 due to viral infection or vaccination could be polyreactive in nature[38–44]. Although polyreactive antibodies are generally considered low-affinity natural antibodies that are protective[38,39], there are studies that have demonstrated the pathogenic potential of polyreactive antibodies[43–45]. In a US study, anaphylactic reaction due to PEG, phosphatidylcholine and other nanoparticle stabilizers in COVID-19 mRNA vaccines was reported in one case per 100,000 doses[46]. This allergic reaction to vaccine stabilizers was supported by positive skin testing for some and IgE detection in a majority of those who demonstrated hypersensitivity reactions to the vaccines[18]. In this study by Mouri M. et al., patients with hypersensitivity to COVID-19 mRNA vaccines were tested for IgE with PEG with a molecular weight of 3000 using ELISA, and PEG with a molecular weight of 2000 using skin testing. The serum samples were diluted 4-fold in phosphate-buffered saline (PBS). The researchers concluded that PEG is antigenic for allergic responses to COVID-19 mRNA vaccines, and that both IgE-mediated and IgG-mediated reactions are potential pathways for this. Therefore, Mouri et al. concluded that the measurement of PEG-specific IgE and IgG could be useful in diagnosing allergy to COVID-19 mRNA vaccines.

Based on several articles[46–50] that dealt with immune response to viruses, we hypothesized that SARS-CoV-2 infection and viral persistence in some individuals, PEG, and other vaccine additives induce Th2 cell biases which, in the presence of host factors, help B cells to become activated and switch their antibody class from IgG to IgE. Considering such a very, very low rate of anaphylaxis to COVID-19 mRNA vaccine compared to relatively such a high level of IgE antibody against different PEGs detected in our study, this may indicate that many PEG antibodies detected in the blood of individuals before and after COVID-19 may not contribute to a hypersensitivity reaction. However, two different studies[51,52] have shown that hypersensitivity due to IgE against PEGylated PEG-asparaginase occurred in 8.7–23.5% of individuals treated with this medicine. In addition to PEG, Mouri et al.[18] also found elevations in both IgG and IgE against polysorbate, which is, like PEG, used in COVID and other vaccines, and which is cross-reactive with PEG. In our earlier studies[53,54], we measured antibodies against phospholipids in the sera of COVID patients with different degrees of severity, and found that autoantibodies targeting phospholipids were detected at a high level in COVID patients, correlating with age and severity of COVID-19. Finally, we showed that monoclonal antibodies made against SARS-CoV-2 reacted strongly with phospholipids and an additional 27 tissue antigens due to cross-reactivity[19,55,56].

Although PEG is a chemical compound and not made of protein, peptides and amino acids, consisting instead of multiple methoxy groups of PEG, studies in animal models[57,58] have shown that PEG becomes highly immunogenic after binding to the ethylene oxide subunits or "backbone." Furthermore, when proteins such as BSA (bovine serum albumin) or KLH (keyhole limpet hemocyanin) are conjugated with multiple PEG molecules and injected into rabbits, they generate antibodies with over 1000-fold higher affinity for multiple PEGs than to hydroxyl-PEG (HO-PEG)-protein that does not contain any methoxy group[59]. This immunogenicity of PEG was shown in humans when they were administered with PEGylated therapeutics. For example, anti-PEG IgM was detected in 46% and anti-PEG IgG was detected in 32% of patients treated with pegaspargase[24,60].

Because of this immunogenicity of multi-PEGs in both animal models and in humans, we sought to explore the possible contribution of anti-PEG antibodies to autoimmunity by applying commercial PEG antibodies against 65 different tissue antigens.

Reaction with mouse monoclonal anti-PEG antibody exhibited a significant reaction with 6 out of the 65 tissue antigens, particularly actin and dsDNA, but showed no reactivity with 59 other tissue antigens (Fig. 6). Rabbit monoclonal anti-PEG antibody demonstrated weaker reactivity with 7 distinct antigens, including phosphatidylcholine, and no reactivity with 58 other tissue antigens (Fig. 7).

To our surprise, the application of rabbit polyclonal anti-PEG antibody resulted in immunoreactivity with 20 out of the 65 tested tissue antigens. The most significant reactions were with phospholipids, actin, myelin-associated glycoprotein (MAG) and TPO. There was no reactivity with 45 other tissue antigens (Fig. 8).

The significant difference between monoclonal and polyclonal anti-PEG antibodies in cross-reactivity with human tissue antigens may stem from their binding affinities. Monoclonal antibodies are highly specific and bind to specific epitopes, whereas polyclonal antibodies can bind to different regions of a particular antigen, better representing the endogenous serum antibody response in individuals[25].

At present, it remains uncertain whether the reaction of polyclonal PEG antibody with so many tissue antigens that are involved in different autoimmune disorders contributes to autoimmune disease. However, the observed reaction of highly PEG-positive human sera with actin as shown in Fig. 5 may offer some support for this idea.

It is possible that the PEG antibody cross-reacted with the tissue antigens shown in our study. The following findings from our study confirm the specificity of PEG antibodies:

1. Simultaneous detection of PEG antibodies against PEG-5 K, PEG-40 K, multi-PEGylated 20 K BSA, PEGylated vaccine components, and phosphatidylcholine in a majority of PEG-positive tested sera.
2. Proportionally to dilution, rabbit polyclonal anti-PEG antibodies exhibited binding to PEG, phospholipids, and actin, but not to myelin oligodendrocyte glycoprotein (MOG), which is among the antigens with which PEG antibody did not react, suggesting selective reactivity.
3. The addition of PEG, phospholipids or actin to the liquid phase of PEG antibody bound to PEG-coated ELISA plates, but not MOG, in proportion to the concentration of an antigen inhibited the binding of PEG-specific antibody to different cross-reactive antigens.

Limitations

Since the final 90 samples significantly positive for SARS-CoV-2 IgG that we used in this study were commercially obtained, we cannot definitively state that this positivity comes from infection with SARS-CoV-2, inoculation with the COVID-19 vaccine, or both, as we do not have medical history for the samples. Future studies that do have accompanying medical history for the tested samples would help to connect antibody levels with the history of exposure to the virus. We also acknowledge the limitation that we used a test that tested for both spike protein and anti-nucleocapsid antibodies together, and future studies can address this limitation.

Conclusions

Our study highlights the significant immunogenicity of PEGylated vaccines and products, demonstrated by elevated IgG, IgA, IgM, and IgE antibodies against PEG. The results of this study demonstrate that PEG-specific antibodies can bind to human tissue antigens, raising important questions about their immunological role. The findings suggest that PEG-specific antibodies:

- Are present in human sera before COVID-19, possibly due to environmental exposure.
- Increase significantly the effects or degrees of post-COVID-19 infection or mRNA vaccination, suggesting induced immune responses.
- May interfere with PEGylated therapeutics, highlighting the need for further research into their clinical implications.

These support the hypothesis that increasing exposure to PEGylated materials in pharmaceuticals, consumer goods, and vaccines contributes to pre-existing and vaccine-induced immune responses to PEG. Understanding the structural nature of PEG antibodies and their potential immunological impact is crucial for optimizing the design of PEGylated drugs and vaccines, as well as mitigating adverse immune reactions in susceptible individuals. Further studies are needed to explore the long-term clinical implications of PEG-related immune responses in vaccinated and unvaccinated individuals.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Received: 18 June 2025; Accepted: 26 August 2025
Published online: 26 September 2025

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Acknowledgements
We would like to acknowledge Joel Bautista for his invaluable assistance in the preparation of this manuscript and the creation of the figures and tables.

Author contributions
A.V. designed the study, performed experiments, monitored experimental quality control, analyzed experimental results, analyzed data, wrote the original manuscript, and helped with editing. E.V. helped in performing the experiments, analyzing data, and editing the manuscript.

Funding
All funding came from the primary author.

Declarations

Competing interests
The authors declare no competing interests.

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