Modulation of the immune response to SARS-CoV-2 vaccination by NSAIDs

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Non-Standard Abbreviation List

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>COX-1</td>
<td>Cyclooxygenase 1, also known as prostaglandin G/H synthase 1</td>
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<tr>
<td>COX-2</td>
<td>Cyclooxygenase 2, also known as prostaglandin G/H synthase 2</td>
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<tr>
<td>CyTOF</td>
<td>Cytometry by Time Of Flight</td>
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<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>NSAIDs</td>
<td>Non-Steroidal Anti-Inflammatory Drugs</td>
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<tr>
<td>OPLS-DA</td>
<td>Orthogonal Projections to Latent Structures Discriminant Analysis</td>
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<tr>
<td>RBD</td>
<td>Receptor Binding Domain</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Severe Acute Respiratory Syndrome CoronaVirus 2</td>
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<td>VAS</td>
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Abstract

Evidence is scarce to guide the use of nonsteroidal anti-inflammatory drugs (NSAIDs) to mitigate SARS-CoV-2 vaccine related adverse effects, given the possibility of blunting the desired immune response. In this pilot study, we deeply phenotyped a small number of volunteers who did or did not take NSAIDs concomitant with SARS-CoV-2 immunizations to seek initial information on the immune response. A SARS-CoV-2 vaccine specific RBD-IgG antibody response and efficacy in the evoked neutralization titers were evident irrespective of concomitant NSAID consumption. Given the sample size, only a large and consistent signal of immunomodulation would have been detectable, and this was not apparent. However, the information gathered may inform the design of a definitive clinical trial. Here, we report a series of divergent omics signals that invite additional hypotheses testing.
Significance Statement

The impact of NSAIDs on the immune response elicited by repeat SARS-CoV-2 immunizations was profiled by immunophenotypic, proteomic and metabolomic approaches in a clinical pilot study of small sample size. A SARS-CoV-2 vaccine specific immune response was evident irrespective of concomitant NSAID consumption. The information gathered may inform the design of a definitive clinical trial.
Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) suppress the formation of prostaglandins (PGs) by blocking the cyclooxygenase activity of the enzymes Prostaglandin G/H Synthases -1 and 2, colloquially known as COX-1 and COX-2 (Ricciotti and FitzGerald, 2011). PGs and related lipids may influence cellular infectivity and are potent modulators of the immune response to viral infection, including by SARS-CoV-2 (Theken et al., 2021). Early concerns that NSAIDs might adversely influence the course of COVID-19 were misplaced (FitzGerald, 2020) and interest has turned to the potential therapeutic benefit of blocking or augmenting the actions of individual PGs (Theken and FitzGerald, 2021).

The emergence of vaccines directed at SARS-CoV-2 prompted conflicting advice – either to take NSAIDs to mitigate the pain and “flu-like” symptoms the vaccines sometimes evoked or to avoid NSAIDs as they might blunt the desired immune response. However, few data underlie the provision of such advice. What information does exist is conflicting, mainly relates to other vaccines, and is based on small trials and anecdotes (Kazama and Senzaki, 2021; Morrison et al., 2022). Here, we decided to seek initial information on the immune response to NSAID consumption concomitant with administration of mRNA vaccine for SARS-CoV-2. In this pilot study, we deeply phenotyped a small number of volunteers who did or did not take NSAIDs at the time of with vaccination. We found that the SARS-CoV-2 vaccine elicited an effective antibody response irrespective of NSAID consumption. Given the sample size, only a large and consistent immunomodulatory signal would have been detectable, and this was not apparent. However, the information gathered in this pilot study may inform the design of a definitive clinical trial.
Materials and Methods

Study population & design

We enrolled five participants at the Institute for Translational Medicine and Therapeutics (ITMAT), University of Pennsylvania, who co-authored this work. Ethics approval for the repeat biosampling protocol (Penn IRB#826459) was granted by the Institutional Review Board of the University of Pennsylvania (FWA00004028; IORG0000029). Informed consent was obtained from all volunteers prior to biosampling. This clinical research study was conducted in accordance with the IRB protocol and relevant guidelines and regulations. Participants met criteria for inclusion (in good health, ≥18 years of age, not pregnant, body weight ≥ 110 pounds) and exclusion (had not received an experimental drug or used an experimental medical device within the past 30 days, blood donation of ≥400 ml in the past 8 weeks). SARS-CoV-2 mRNA vaccines were administered in the vaccine clinics of the Perelman School of Medicine, University of Pennsylvania under the Emergency Use Authorizations from the Food and Drug Administration. None of the consented participants dropped out.

Participants shared the time and date of their self-scheduled mRNA vaccination (Moderna Spikevax®, mRNA-1273 or the Pfizer-BioNTech Comirnaty®, BNT162b2) to allow timed biosampling (whole blood collections drawn from the median cubital vein via venipuncture using a 22 G butterfly needle, BD, Franklin, Lakes, NJ, USA, and urine samples) at baseline, and at 1hr, 4hr, 1 week, 2 weeks, 4 weeks, 3 months, and 6 months after each repeat vaccination. The 3rd immunization was investigated using the same sampling scheme with the exception that the 1-hour post vaccination time point was replaced by a +24-hour time point. Some participants decided to self-medicate with 400 mg over-the-counter ibuprofen orally at approximately 1, 5 and 12 hours after having received the SARS-CoV-2 vaccination. Adverse effects were self-reported using a specified symptom catalog (Publication) extended by a visual analog scale to capture localized pain experienced at the injection site. We collected this information to parse the data set into two groups, “vaccination” and “vaccination with anti-inflammatory self-
medication”, labeled subsequently as “Vaccine” (or “control”) and “Vaccine & NSAID” (as shown in Figure 1A).

**Urinary PG analysis**

Urine samples were provided at each blood draw visit and were frozen at -80°C until analysis. Urinary prostanoid metabolites were quantified using ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) as previously described (Li et al., 2018). Systemic synthesis of PGE₂ was determined by quantifying its major urinary metabolite 7-hydroxy-5, 11-diketotetranorprostane-1, 16-dioic acid (PGEM) and results were normalized to urinary creatinine. Unlabeled and deuterated analogs of PGEM (PGEM-d6) and Creatinine (Creatinine-d3) were purchased from Cayman Chemical.

**SARS-CoV-2 IgG and IgM ELISA assays**

The enzyme-linked immunosorbent assays (ELISAs) were conducted as described previously (Amanat et al., 2020; Flannery et al., 2020) with the following modifications. All samples were initially tested in duplicate at a serum dilution of 1:50. Samples with an IgG or IgM concentration less than the limit of detection (0.20 arbitrary units) were repeated using at least a seven-point dilution series to attain quantitative results. The IgM response remained unchanged close to the lower limit of detection (results not shown).

**Production of vesicular stomatitis virus (VSV) pseudotypes of SARS-CoV-2 S:**

Production of vesicular stomatitis virus (VSV) pseudotypes with the SARS-CoV-2 spike (S) occurred as follows: 293T cells were plated for 24 h at 5 x 10⁶ cells per 10 cm dish and were then transfected using calcium phosphate with 35 μg of pCG1 SARS-CoV-2 D614G Δ18 expression plasmid that encodes for a codon optimized SARS-CoV-2 S gene with an 18-residue truncation in the cytoplasmic tail. To increase expression of the transfected DNA, the culture media were replaced 12 h post transfection with media containing 5mM sodium butyrate. Then, 30 h post transfection, the SARS-CoV-2 S-expressing cells were infected for 2-4 hours with VSV-G pseudotyped VSVΔG-RFP at a multiplicity of infection (MOI) of ~1-3. After infection, cells were washed twice with media
to remove unbound virus. The media containing the VSVΔG-RFP SARS-CoV-2 pseudotypes were harvested 28-30 hours post infection. The media was then centrifuged twice at 6,000 x g and aliquoted and stored at -80°C until they were used in antibody neutralization analysis.

**Antibody neutralization assay using VSVΔG-RFP SARS-CoV-2:**

Sera were heat-inactivated for 30 min at 55°C prior to use in the assay. Vero E6 that stably expressed TMPRSS2 were seeded in 100 μl at 2.5 x 10^4 cells/well in a collagen coated 96-well plate. The following day, two-fold serially diluted serum samples were mixed with VSVΔG-RFP SARS-CoV-2 pseudotype virus at 100-300 focus forming units per well and incubated at 37 °C for 1 h. Additionally, 1E9F9, a mouse anti-VSV Indiana G (Ab01402-2.0: Absolute Antibody, Boston, MA, USA), was added at a concentration of 600 ng/ml to neutralize any potential VSV-G carryover. The serum-virus mixture was then used to replace the media on the TMPRSS2 expressing VeroE6 cells. At 22 h post infection, the cells were washed and fixed with 4% paraformaldehyde and visualized on a S6 FlouroSpot Analyzer (CTL, Shaker Heights, OH, USA). The individual infected foci were counted, and the number was compared to control wells lacking the antibody. The focus reduction neutralization titer 50% (FRNT₅₀) was measured as the greatest serum dilution at which the focus count was reduced by at least 50% relative to control cells that were infected with pseudotype virus in the absence of human serum. At least two technical replicates were performed to determine the FRNT₅₀ titers for each sample, which were reported for each sample as a geometric mean.

**Whole blood mass cytometry**

A 300 μl sample of whole blood collected from the median cubital vein in heparinized vacutainers® (33 IU; BD, Franklin Lakes, NJ, USA) was added to a Maxpar® tube (Fluidigm, San Francisco, CA, USA) (Petes et al., 2022). Then 420 μl of PROT1 proteomic stabilizer (SMART TUBE INC, Las Vegas, NV, USA) was added and gently mixed before incubating at room temperature in the dark for 10 min. The samples were then frozen at -80°C until further analysis. One day before data acquisition, samples were prepared using the protocol from the thaw-lyse buffer (SMART TUBE INC, Las
Samples were thawed at 4°C for 30 min and were mixed with 1X of the thaw lyse buffer for 10 min at room temperature. Samples were spun down and subsequently incubated with 25 ml of 1X thaw lyse buffer for 10 min at room temperature. Samples again were pelleted and were resuspended in 1.6% PFA with Iridium overnight at 4°C. Prior to data acquisition, cells were washed twice in PBS and once in dH₂O were added to 96-well plates in duplicate.

**Olink immune response analysis**

Proteomic profiling of participant blood plasma samples was conducted using the Olink® Target 96 Immuno-Oncology platform (Olink Proteomics, Boston, MA, USA). In total, 20μl of plasma samples were added to 96-well plates in duplicate. The plates were frozen in dry ice, shipped, and analyzed by Olink Proteomics.

**Metabolomics**

Urine samples were analyzed using NMR spectroscopy as previously described (Sengupta and Weljie, 2019).

**Statistical Analyses**

To discern signal from noise, repeated measures where available were aggregated by time-point and condition. Plasma proteomics, urine metabolomics and plasma cytometry were not available for the 3rd immunization. Data from urinary PGEM, plasma RBD-IgG, and neutralization assays were baseline-normalized within each subject and immunization. These normalized values were tested for differences between vaccine cohorts at each timepoint using the nonparametric Mann-Whitney U Test (using the wilcox.test function in R v4.2.1). Given the known ibuprofen drug effects, the urinary PGEM data were analyzed with a one-sided test, while the plasma RBD-IgG and neutralization assay data were analyzed with two-sided tests. Additionally, the area under the curve (AUC) was calculated for each subject’s urinary PGEM time course using the trapezoid method. AUC values from each cohort were compared using a one-sided Mann-Whitney U test (results not shown), which supported the results obtained from the time point-specific test. Test results yielding p-values < 0.05 were considered
significant. Given the exploratory nature of these analyses, p-values were not corrected for multiple testing.

Cytometry data were analyzed for immune cell populations in the OMIQ software from Dotmatics (www.omiq.ai, www.dotmatics.com) using the gating strategy shown in Figure S 1. Cell population data were exported from OMIQ. Cell populations were converted to percent of parent cell population and normalized as fold-change from baseline to elucidate potential vaccine- and/or NSAID-related effects.

Statistical analyses on the proteome were performed per Analysis of Variance included in the Olink Insights Stat Analysis (Olink). For the metabolomics, each spectrum was binned (0.001 ppm) and integrated to generate the data matrix. The spectra were normalized to account for any sample dilution related issues. Data were analyzed using multivariate and univariate statistics as described previously (Sengupta and Weljie, 2019).
Results

Data Structure

The data set consisted of \( n=5 \) instances for the vaccine cohort and \( n=6 \) instances for the vaccine & NSAID cohort stratified by number of repeat immunizations as shown in Figure 1A. **NSAIDs showed anticipated drug effects**

As expected, PG biosynthesis was suppressed by ibuprofen per visual inspection at the 4- and 24-hour time points post immunization but given the high variability within and between individuals this did not attain statistical significance (Figure 1B). Mean urinary normalized PGEM concentrations 4 hours post immunization were depressed in participants on NSAIDs (6.0±1.1 ng/mg creatinine) compared to controls (9.5±2.6 ng/mg creatinine; \( p=0.17 \) one-sided Mann-Whitney U test; Figure 1B left & center). Urinary PGE-M fell to 2.1±1.2 ng/mg creatinine in participants on NSAIDs compared to 7.5±3.4 ng/mg creatinine in controls by 24 hours post immunization (\( p=0.2 \) one-sided Mann-Whitney U test). Prior to the 2nd immunization, both groups had comparable baseline PGEM concentrations of 9.3±6.1 ng/mg creatinine and 10.4±2.3 ng/mg creatinine, respectively. Again, baseline urinary PGE-M levels before the 3rd immunization were similar in both groups (8±1.7 ng/mg creatinine and 7.9±2.2 ng/mg creatinine, respectively).

Common expected adverse effects included redness, swelling and pain localized at the injection side, as well as fatigue, headache, and chills. Body temperature stayed within normal range. The incidence of self-rating these adverse effects as mild, moderate, or severe showed a high degree of variability. However, the central tendency of the time courses suggests an overall lower adverse effect profile in the individuals on NSAIDs (Figure 1B bottom left). In the visual analog scale (VAS) ratings of pain at the injection site both groups reported similar mean intensities except for the 4-hour timepoint where individuals on NSAIDs self-reported less pain compared to controls (1.7±1.7 VAS and 3.7±1.5 VAS, respectively; Figure 1B bottom right).

Overall, these results support that NSAIDs showed both pharmacological and clinical signals consistent with a mitigation of the adverse effect profile post immunization.
**SARS-CoV-2 vaccine specific antibody response mounted irrespective of NSAIDs**

Average RBD IgG concentrations were lower in participants on NSAIDs compared to controls. This divergence trended to be statistically significant at the 1 week and 2 weeks’ time points following vaccination (10.0±3.2 versus 28.5±7.9 fold-change from baseline, p=0.067; and 16.5±4.6 versus 42.3±9.8 fold-change from baseline, p=0.048, respectively, two-sided Mann-Whitney U test). Reaching average peak RBD IgG concentrations was delayed in participants on NSAIDs compared to controls. The peak 20.2±9.2 fold-change from baseline in participants on ibuprofen noted at 4 weeks post immunization was less than the peak 42.3±9.8-fold-change from baseline observed in the controls at 2 weeks after vaccination (Figure 1C). On the other hand, NSAID intake did not appear to significantly alter virus neutralization titers. A mean peak 69.3±39.4 fold-change from baseline was observed in participants on ibuprofen compared to a 59.0±24.8 fold-change from baseline in the controls at the 2 and 4-week time point post immunization, respectively (Figure 1D). Overall, these data suggest that a SARS-CoV-2 vaccine specific antibody response was mounted irrespective of ibuprofen co-administration.

**Shifts in immune cell populations were reflective of vaccination and NSAID effects**

Cell populations of eosinophils normalized to granulocytes showed small time-dependent increases in the NSAID group compared to controls (Figure 2A left) reminiscent of NSAID-associated eosinophilia reported previously (Choudhary et al., 2013). Similarly, marginal changes in neutrophil populations normalized to total granulocytes were noticeable (Figure 2A right), as expected (Strom et al., 1993).

A divergent signal was noted in the B-cell populations normalized to the CD45+ cell population (Figure 2B). Four hours post immunization B-cells were mostly unchanged in participants on ibuprofen (0.1-fold decrease from baseline) compared to the 0.6-fold from baseline increase in the controls. The population of B cells that were plasmablasts was evident at 1 week post immunization amounting to a 0.8-fold increase from baseline across pooled participants (Figure 2C left). This time-specific signal is in line with a similar effect reported in 145 subjects receiving the BNT162b2-BioNTech/Pfizer...
vaccine (Ciabattini et al., 2021). Unexpectedly, here participants on ibuprofen uniformly showed a more pronounced expansion (1.3-fold increase from baseline) than observed in the controls (0.3-fold increase from baseline, Figure 2C right). In addition, we detected a decline in δγ T-cells normalized to the alpha-beta-T-cell (αBT) population at 2- and 4-weeks post immunization in participants on ibuprofen compared to the controls (Figure 2D).

Taken together, these data suggest a plasmablast expansion in response to vaccination and a depletion in δγ T cells following concomitant ibuprofen administration.

**Immunomodulatory response in the proteome**

Interleukin-6 (IL6) showed significant time-specific variance (ANOVA, F-test adjusted p-value=0.002), as expected (Bergamaschi et al., 2021), with an upregulation 4 hours after vaccination compared to other time points (post-hoc tests, adjusted p-values <0.001) for all participants (Figure 3A top left). Several proteins, such as PRKCQ, IRAK4, and DAPP1 showed a trend toward lower values in participants on ibuprofen compared to controls (Figure 3A).

Prompted by initiatives to identify host factors potentially modulating the viral life cycle, we found that three out of 92 proteins had a significant difference in means between participants on ibuprofen compared to controls (Figure 3B top, irrespective of the temporal order of the biosamples). These proteins were “PC4 and SFRS1-interacting protein” (PSIP1, mean normalized protein expression or NPX of 3.72 and 2.73, respectively, adjusted p-value=0.0021, Figure 3B bottom left), “Coxsackievirus and adenovirus receptor” (CXADR, mean NPX of 2.54 and 2.28, respectively, adjusted p-value=0.0193) and “Interferon lambda receptor 1” (IFN-λ, mean NPX of 2.60 and 2.83, respectively, adjusted p-value=0.0371, Figure 3B bottom right). PSIP1 was identified as a high-confidence interacting protein (HCIPs) in the characterization of the host and SARS-CoV-2 protein–protein interaction network (Chen et al., 2021). This screen suggested that PSIP1 regulates transcription during the life cycle of a SARS-CoV-2 viral infection through interaction with NSP3, a viral protein essential for its replication and transcription (Lei et al., 2018).
Summarizing these results, an IL-6 signal highlighted the immunomodulatory response following the SARS-CoV-2 vaccination, while diverging trends in PRKCQ, IRAK4, and DAPP1 offer candidates to evaluate potential modulation by NSAIDs in future investigations. The observation of the two-fold difference in PSIP1 abundance in vivo opens an opportunity to scale the virus-host protein interactions from cellular models into humans.

**Metabolomics data**

The OPLS-DA scores plot showed discrimination in global metabolic profile between the control and NSAID conditions with a $Q^2$, the cross validated $R^2$, of 19% (CV-ANOVA $p<0.02$) (Figure 4A). Taurine (bins 3.243 – 3.245) was among the prominent identified metabolites that was significantly different between the two treatment cohorts ($q<0.2$ by t test) (Figure 4B left). However, the time-course data did not indicate directional changes between the control and NSAID conditions (Figure 4B right).
Discussion

This study determined that a SARS-CoV-2 vaccine specific immune response occurred in a small number of apparent healthy volunteers irrespective of NSAID intake. While the capacity of the mounted antibody response to neutralize the SARS-CoV-2 virus *ex vivo* achieved comparable levels in both groups, the quantified response for SARS-CoV-2-specific RBD IgG measured by ELISA showed a transient dampening in the NSAID group compared to controls. With an eye toward evaluating these findings in future studies, we performed a sample size estimate. Briefly, we calculate a sample size of n=90 per group based on a one-tailed test at alpha=0.01 and beta=0.05 will be needed. Using the IgG ELISA at 1 week post 2nd immunization, specifically the log of the fold-change to baseline, the effect size was estimated to be 1.59; and the standard deviation was estimated to be 0.91. Using these estimates, we performed a power calculation based on the two-sample T-test. To be conservative, we doubled the standard deviation estimate and halved the effect size estimate. We did not consider sex- and age-dependent immunomodulatory effects in our calculations at this stage since we did not observe an obvious impact on the response of any of the variables. This underscores the value of using preliminary data to show feasibility of conducting a clinical trial powered to produce a definite answer.

A single timepoint study in several hundred healthcare workers found no evidence that sex or ethnicity modulated the SARS-CoV-2-specific IgG titers 21 days after the first BNT162b2 vaccine dose, but older individuals generated significantly lower titers (Abu Jabal et al., 2021). A larger study to assess protection against SARS-CoV-2 infection after the second vaccine dose (BNT162b2) in 21,000 participants did not reveal effects specific to sex, ethnicity or age (Polack et al., 2020). A meta-analysis in 67,000 recipients of either BNT162b2-BioNTech/Pfizer, 1273-Moderna, Ad26.COV2.S-Johnson&Johnson/Janssen or Gam-COVID-Vac-Gamaleja demonstrated a higher effectiveness of preventing COVID-19 in men compared to women (Bignucolo et al., 2021). However, reporting bias might have skewed this observation (Jensen et al., 2022) given that we would expect a more robust immune and clinical response in women compared to men. This speculation is based on the overall higher responsiveness evident to viral vaccines in women compared to men across the full
spectrum of the immune response. Dendritic cells and macrophages are more active in women compared to men during the innate immune response resulting in a differential profile of cytokines and chemokines released. This drives expansion of B and T cells with sex-specific divergence during the early phase of the adaptive immune response followed by a more pronounced T-helper-2 (Th2), cytokine and humoral response in women than men during the late adaptive phase (Klein et al., 2010; Klein and Flanagan, 2016).

The transient upregulation of IL6 four hours after vaccination in both groups suggests a vaccine-related signal (Karwaciak et al., 2021) unhampered by NSAIDs. Interestingly, upon further visual inspection, this IL6 signal clustered with NF2 which showed a similar time course albeit at much higher variance (p>0.05 two-sided Wilcoxon rank sum exact test; Figure 5 A, B). NF2 (Neurofibromin 2, Merlin) is mostly known for its association with neurofibromatosis type II in patients with mutations in this gene. Downregulation of NF2 is known to compromise its tumor-suppressive characteristics and has recently been suggested as a biomarker for COVID-19 (Patel et al., 2021) likely due to its involvement in cell-cell adhesion and transmembrane signaling (Curto and McClatchey, 2008). Upregulation of NF2, in contrast, and the relevance of downstream effects is unclear. Two candidates with differential abundance at the four-hour time point stand out per visual inspection, CXCL12 clustered with PIK3AP1 (Figure 5 A, B, C). In both cases, abundances are selectively lower for this time point in the NSAID group compared to the one in controls (p=0.2 for CXCL12 and p=0.4 for PIKAP1, two-sided Wilcoxon rank sum exact test for the 4-hour timepoint), suggesting that this trend might represent an ibuprofen-induced effect. CXCL12 (stromal cell-derived factor-1, SDF-1) is a chemokine critical for lymphocytes and monocyte (Bleul et al., 1996) and its localized expression can be suppressed ex vivo by NSAIDs and steroids (Kim et al., 2006). Upregulation of CXCL12 has been reported during the course of a SARS-CoV-2 infection (Khalil et al., 2021). Here, a testable hypothesis is whether chronic NSAID intake would lead to a sustained suppression of CXCL12 and whether this associates with the plasticity of immune cells.
The divergent response of PRKCQ, IRAK4, and DAPP1 to NSAIDs in the immune response proteome pointed to mechanisms of potential relevance, though this is coupled with substantial uncertainty. PRKCQ, for example, is indicative for immunogenicity through its role in T-cell activation (Uniprot) and is suppressed by NSAIDs (Paccani et al., 2002). IRAK4 boosts the immune response through NF-kappaB-mediated activation of Toll-like receptor (TLR) and T-cell receptor (TCR) signaling pathways (Uniprot) and is under evaluation as a potential anti-inflammatory drug target (Khanfar and Alqtaishat, 2019). DAPP1 regulates the signaling of B-cell antigen receptors (Wang et al., 2011), where the adaptive cellular response is possibly curbed by NSAIDs (Bancos et al., 2009). We see this as a prompt to explore this in larger studies, for example, in the UK Biobank (Olink).

Similarly, the decline in δγ T-cells associated with NSAIDs in the present study is of potential interest. Previous reports suggested that the COX-1 specific inhibitor, bufexamac, suppressed δγ T cell function in PBMCs ex vivo (Inaoka et al., 2006). Here, the question remains if a change in immune cell population translates to a functional impact.

The strength of this study is the longitudinal deep phenotyping covering several cycles of SARS-CoV-2 immunizations in each participant. The limitation is the small number of volunteers. The findings of our pilot study can be useful to frame the design of a clinical study powered to answer the question definitively whether NSAIDs do indeed interact with the immune response to SARS-CoV-2 immunization to a clinically relevant extent.
Authorship Contributions

Participated in research design: Skarke C, Lordan R, Barekat K, Naik A & FitzGerald GA


Contributed new reagents or analytic tools: Bates P & FitzGerald GA

Performed data analysis: Skarke C, Lordan R, Greenplate AR, Grant GR, Lahens NF & FitzGerald GA

Wrote or contributed to the writing of the manuscript: Skarke C, Lordan R, Prak ETL & FitzGerald GA
References


Publication Symptom Catalog, Pfizer-BioNTech COVID-19 Vaccine EUA Fact Sheet for HCP rev 010621


Uniprot PRKCQ (Protein kinase C theta type), in, [https://www.uniprot.org/uniprotkb/Q04759/entry](https://www.uniprot.org/uniprotkb/Q04759/entry).

Footnotes

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Legends for Figures

Figure 1

(A) Study design. A group of n=5 participants received repeat immunizations for SARS-CoV-2. Please note that four subjects (2 times n=2) were part of this study for their 2nd and 3rd immunization, while one subject (young male, last row) cycled through the 1st, 2nd and 3rd immunization. Type of vaccine indicated by B for BioNTech and M for Moderna with self-medicated ibuprofen marked in red.

(B) Pharmacological and clinical effects in individuals on self-medicated NSAIDs (red) compared to controls (blue) with readouts in prostaglandin E metabolite (PGEM) from timed spot urine collections (top), profile for common, expected adverse effects rated as either mild, moderate, or severe (bottom left) and ratings of pain at the injection site on a visual analog scale (0-10 scale; 0=no pain; 10=maximum pain; bottom right). Data from all immunizations were aggregated for each time point.

(C) SARS-CoV-2 specific RBD-IgG antibody response in individuals on self-medicated NSAIDs (red) compared to controls (blue). Data from all immunizations were aggregated for each time point. * p=0.048, two-sided Mann-Whitney U test for the comparison at two weeks’ time point following vaccination. Note that the comparison at one week time point following vaccination trended with, p=0.067 in the two-sided Mann-Whitney U test.

(D) The response in the SARS-CoV-2 evoked neutralization titers in individuals on self-medicated NSAIDs (red) compared to controls (blue). Data from all immunizations were aggregated for each time point.

Figure 2

Abundances in immune cells in individuals on self-medicated NSAIDs (red) compared to controls (blue) for eosinophils and neutrophils (A), CD45 positive immune cells (B), B-cells (C right) and δγT cells (D). Aggregated data on B-cell abundances from both groups displayed in (C left).
Figure 3
(A) Abundances of proteins depicted in individuals on self-medicated NSAIDs (red) compared to controls (blue) for IL6, PRKCQ, IRAK4 and DAPP1.
(B) Screening 92 preselected candidate proteins in the Olink immune response panel, the proteins PSIP1, CXADR and IFNLR1 showed a significant difference in means between participants on NSAIDs compared to controls (top) with boxplot distribution shown for IFNLR1 and PSIP1 (bottom).

Figure 4
Discrimination of the global metabolic profile per OPLS-DA scores plot in individuals on self-medicated NSAIDs compared to controls (A) and taurine shown as boxplot distribution (B left) and over time (B right).

Figure 5
(A) Heatmap of the baseline normalized Olink metabolite panel aggregated for time point and condition. Selected proteins with directional (IL6, NF2) and opposing (CXCL12, PIK3AP1) transient effects are highlighted in (B). The time course of CXCL12 abundance is shown in (C) to highlight the differential signal at the four-hour time point between vaccinated participants on NSAID compared to control.
Figure 1

(A) Not-So-Young, 61.8±9.3 yrs, male, n=2
Young, female, n=2
Young, male, n=1

(B) Aggregate of 1st, 2nd & 3rd Immunization

(C) RBD-IgG Titers

(D) Neutralization Titers
Figure 2

(A) Eosinophils

(B) Neutrophils

[Graphs showing changes in populations of eosinophils and neutrophils over time with and without NSAID treatment.]

(B) B-cells

[Graph showing changes in the population of B-cells over time with and without NSAID treatment.]

(C) Plasmablasts

[Graph showing changes in the population of plasmablasts over time with and without NSAID treatment.]

(D) gdTCells

[Graph showing changes in the population of gdTCells over time with and without NSAID treatment.]

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Figure 3

(A) IL6 and PRKCIQ levels from baseline:
- Vaccine
- Vaccine&NSAID

(B) 
- PSIP1
- CXADR
- IFNLR1

NPX\text{Vaccine} - NPX\text{Vaccine&NSAID}

- IFNLR1
  - Vaccine
  - Vaccine&NSAID

- PSIP1
  - Vaccine
  - Vaccine&NSAID
Figure 4

(A) 

(B)
Figure 5

(A) Olink Proteins

(B) Selected Olink Proteins

(C) CXCL12

[Diagram depicting various proteins and their fold change over time with Vaccine and Vaccine&NSAID treatments.]