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Modulation of the Immune Response to Severe Acute Respiratory Syndrome Coronavirus 2 Vaccination by Nonsteroidal Anti-Inflammatory Drugs


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ABSTRACT

Evidence is scarce to guide the use of nonsteroidal anti-inflammatory drugs (NSAIDs) to mitigate severe acute respiratory syndrome coronavirus 2 (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 receptor binding domain (RBD) IgG antibody response and efficacy in the evoked neutralization titers were evident irrespective of concomitant NSAID consumption. Given the small 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 invites additional hypotheses testing.

SIGNIFICANCE STATEMENT

The impact of nonsteroidal anti-inflammatory drugs (NSAIDs) on the immune response elicited by repeat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunizations was profiled by immunophenotypic, proteomic, and metabonomic 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 (COX) 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 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The impact of NSAIDs on the immune response to SARS-CoV-2 vaccination is largely unknown.

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coronavirus 2 (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 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 and Design. We enrolled five participants at the Institute for Translational Medicine and Therapeutics (ITMAT), University of Pennsylvania, who coauthored this work. The Institutional Review Board (IRB) of the University of Pennsylvania (FWA00004028; IORG0000029) granted ethics approval for the repeat biosampling protocol (Penn IRB#826459). 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, receiving a blood donation of ≥400 ml in the past 8 weeks). SARS-CoV-2 mRNA vaccines were administered in the vaccine clinics of the University of Pennsylvania Perelman School of Medicine under the Emergency Use Authorizations from the Food and Drug Administration. None of the consented participants withdrew from the study.

Participants shared the time and date of their self-scheduled mRNA vaccination (Moderna Spikevax (mRNA-1273) or 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) and urine samples] at baseline and at 1 hour, 4 hours, 1 week, 2 weeks, 4 weeks, 3 months, and 6 months after each repeat vaccination. The third immunization was investigated using the same sampling scheme with the exception that the 1-hour postvaccination 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 receiving the SARS-CoV-2 vaccination. Adverse effects were self-reported using a specified symptom catalog (https://s3.wp.wsu.edu/uploads/sites/30/2021/03/Pfizer-BioNTech-COVID-19-Vaccine-EUA-Fact-Sheet-for-HCP-rev-010621.pdf) 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” (control) and “Vaccine & NSAID” (as shown in Fig. 1A).
Urinary PG analysis. Urine samples were provided at each blood draw visit and were frozen at –80°C until analysis. Urinary prostaglandin metabolites were quantified using ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) as previously described (Li et al., 2018). Systemic synthesis of prostaglandin E2 (PGE2) was determined by quantifying its major urinary metabolite 7-hydroxy-5,11-diketotetranorprostan-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 Enzyme-Linked Immunosorbent 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 Pseudotypes of SARS-CoV-2 Spike. Production of vesicular stomatitis virus (VSV) pseudotypes with the SARS-CoV-2 spike (S) occurred as follows: 293T cells were plated for 24 hours at 5 x 10^5 cells per 10-cm dish and were then transfected using calcium phosphate with 35 μg of pC1G 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 optimized SARS-CoV-2 S gene with an 18-residue truncation in the cytoplasmic tail. The samples were then frozen at –80°C until they were used in antibody neutralization assay.

Antibody Neutralization Assay Using VSV-G-RFP SARS-CoV-2. Sera were heat inactivated for 30 minutes at 55°C prior to use in the assay. Vero E6 cells that stably expressed TMPRSS2 were seeded in 100 μl at 2.5 x 10^4 cells per well in a collagen-coated 96-well plate. The following day, 2-fold serially diluted serum samples were mixed with VSV-G pseudotyped VSV-G-Red Fluorescent Protein (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 VSV-G-RFP SARS-CoV-2 pseudotypes were harvested 28–30 hours postinfection. The media was then centrifuged twice at 6000 x g and aliquoted and stored at –80°C until they were used in antibody neutralization analysis.

Results

Data Structure. The data set consisted of n = 5 instances for the vaccine cohort and n = 6 instances for the vaccine and NSAID cohort stratified by number of repeat immunizations as shown in Fig. 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 postimmunization, but given the high variability within and between individuals, this did not attain statistical significance (Fig. 1B). Mean urinary normalized PGEM concentrations 4 hours postimmunization were depressed in participants on NSAIDs (6.0 ± 1.1 ng/mg creatinine) compared with controls (9.5 ± 2.6 ng/mg creatinine),
Urinary PGE-M fell to $2.1 \pm 1.2$ ng/mg creatinine in participants on NSAIDs compared with $7.5 \pm 3.4$ ng/mg creatinine in controls by 24 hours postimmunization ($P = 0.17$, one-sided Mann-Whitney $U$ test; Fig. 1B, left and center). Urinary PGE-M fell to $2.1 \pm 1.2$ ng/mg creatinine in participants on NSAIDs compared with $7.5 \pm 3.4$ ng/mg creatinine in controls by 24 hours postimmunization ($P = 0.17$, one-sided Mann-Whitney $U$ test). Prior to the second immunization, both groups had comparable baseline PEG concentrations of $9.3 \pm 6.1$ ng/mg creatinine and $10.4 \pm 2.3$ ng/mg creatinine, respectively. Again, baseline urinary PGE-M levels before the third immunization were similar in both groups ($8 \pm 1.7$ ng/mg creatinine and $7.9 \pm 2.2$ ng/mg creatinine, respectively).

Common expected adverse effects included redness, swelling, and pain localized at the injection site 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 (Fig. 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 time point where individuals on NSAIDs self-reported less pain compared with controls ($1.7 \pm 1.7$ VAS and $3.7 \pm 1.5$ VAS, respectively; Fig. 1B, bottom right).

Overall, these results support that NSAIDs showed both pharmacological and clinical signals consistent with a mitigation of the adverse effect profile postimmunization.

**SARS-CoV-2 Vaccine-Specific Antibody Response Mounted Irrespective of NSAIDs.** Average RBD IgG concentrations were lower in participants on NSAIDs compared with controls. This divergence trended to be statistically significant at the 1-week and 2-week time points after vaccination ($10.0 \pm 3.2$ vs. $28.5 \pm 7.9$-fold change from baseline ($P = 0.067$) and $16.5 \pm 4.6$ vs. $42.3 \pm 9.8$-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 with controls. The peak $20.2 \pm 9.2$-fold change from baseline in participants on ibuprofen noted at 4 weeks postimmunization was less than the peak $42.3 \pm 9.8$-fold change from baseline observed in the controls at 2 weeks after vaccination (Fig. 1C). On the other hand, NSAID intake did not appear to significantly alter virus neutralization titers. A mean peak $69.3 \pm 39.4$-fold change from baseline was observed in participants on ibuprofen compared with a $59.0 \pm 24.8$-fold change from baseline ($P = 0.2$ one-sided Mann-Whitney $U$ test). The peak $69.3 \pm 39.4$-fold change from baseline was observed in participants on ibuprofen compared with a $59.0 \pm 24.8$-fold change from baseline ($P = 0.2$ one-sided Mann-Whitney $U$ test). The peak $69.3 \pm 39.4$-fold change from baseline was observed in participants on ibuprofen compared with a $59.0 \pm 24.8$-fold change from baseline ($P = 0.2$ one-sided Mann-Whitney $U$ test).

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 across pooled participants (Fig. 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 across pooled participants (Fig. 2C, left). In addition, we detected a decline in gamma-delta T cells normalized to the alpha-beta T cell population at 2 weeks and 4 weeks postimmunization in participants on ibuprofen compared with the controls (Fig. 2D).

Taken together, these data suggest a plasmablast expansion in response to vaccination and a depletion in $\gamma\delta$ T cells after concomitant ibuprofen 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 with controls (Fig. 2A, left) and eosinophils and neutrophils (A), CD45-positive immune cells (B), B cells (C, right), and $\delta\gamma$ T cells (D). Aggregated data on B cell abundances from both groups displayed in (C, left).

**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 with other time points (post hoc tests, adjusted \( P \) values < 0.001) for all participants (Fig. 3A, top left). Several proteins such as protein kinase C theta (PRKCQ), interleukin-1 receptor-associated kinase 4 (IRAK4), and dual adaptor for phosphotyrosine and 3-phosphoinositides 1 (DAPP1) showed a trend toward lower values in participants on ibuprofen compared with controls (Fig. 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 NSAIDs compared with controls (Fig. 3B, top, irrespective of the temporal order of the biosamples). These proteins were PC4 and SFRS1-interacting protein (PSIP1; mean normalized protein expression (NPX) of 3.72 and 2.73, respectively; adjusted \( P \) value = 0.0371; Fig. 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-\( \lambda \); mean NPX of 2.60 and 2.83, respectively; adjusted \( P \) value = 0.0371; Fig. 3B, bottom right). PSIP1 was identified as a high-confidence interacting protein (HCIP) 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 IL6 signal highlighted the immunomodulatory response after the SARS-CoV-2 vaccination, whereas diverging trends in PRKCQ, IRAK4, and DAPP1 offer candidates to evaluate potential modulation by NSAIDs in future investigations. The observation of the 2-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 orthogonal projections to latent structures discriminant analysis (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 \( P < 0.02 \)) (Fig. 4A). Taurine (bins 3.243–3.245) was among the prominent identified metabolites that were significantly different between the two treatment cohorts (q < 0.2 by \( t \) test) (Fig. 4B, left). However, the time course data did not indicate directional changes between the control and NSAID conditions (Fig. 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. Although 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 with controls. With an eye toward evaluating these findings in future studies, we performed a
The sample size estimate. Briefly, we calculate that 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 after second 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 time point 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), mRNA-1273 (Moderna), Ad26.COV2.S (Johnson & Johnson/Janssen), or Gam-COVID-Vac (Gamaleya) demonstrated a higher effectiveness of preventing COVID-19 in men compared with 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 with men. This speculation is based on the overall higher responsiveness evident to viral vaccines in women compared with men across the full spectrum of the immune response. Dendritic cells and macrophages are more active in women compared with 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 4 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 neurofibrin 2 (NF2), which showed a similar time course, albeit at much higher variance ($P > 0.05$ two-sided Wilcoxon rank sum exact test; Fig. 5, A and B). NF2 (neurofibrin 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 4-hour time point stand out per visual inspection: C-X-C motif chemokine ligand 12 (CXCL12) clustered with phosphoinositide 3-kinase adapter protein 1 (PIK3AP1) (Fig. 5, A–C). In both cases, abundances are selectively lower for this time point in the NSAID group compared with the one in controls ($P = 0.2$ for CXCL12 and $P = 0.4$ for PIK3AP1, two-sided Wilcoxon rank sum exact test for the 4-hour time point), suggesting that this trend might represent an ibuprofen-induced effect. CXCL12, also known as stromal cell–derived factor 1 (SDF-1), is a chemokine critical for lymphocytes and monocytes (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 provides opposite to mechanisms of potential relevance, although this is coupled with substantial uncertainty. PRKCQ, for example, is indicative for immunogenicity through its role in T cell activation (https://www.uniprot.org/uniprotkb/Q04759/entry) 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 (https://www.uniprot.org/uniprotkb/Q9NW3Z3/entry) and is under evaluation as a potential anti-inflammatory drug target (Khanfar and Aqltaishat, 2019). DAPPI 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 (https://o link.com/news/uk-biobank-pharma-proteomics-project-extended-to-explore-3072-platform/).

Similarly, the decline in $\delta^{\gamma}$ T cells associated with NSAIDs in the present study is of potential interest. Previous reports suggested that the COX-1 specific inhibitor bexufamac suppressed $\delta^{\gamma}$ T cell function in peripheral blood mononuclear cells (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 definitively answer the question of whether NSAIDs do indeed interact with the immune response to SARS-CoV-2 immunization to a clinically relevant extent.

**Authorship Contributions**

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