Received: 2 May 2022 | Revised: 3 June 2022 | Accepted: 7 June 2022

DOI: 10.1111/all.15405

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

LETTER

Immune-inflammatory proteome of elite ice hockey players before and after SARS-CoV-2 infection

To the Editor

Coronavirus disease 19 (COVID-19) is an infectious disease transmitted mainly through aerosol spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and in most cases leads mild to moderate respiratory illness, which usually resolves within 5–7 days.[1] Regular moderate-to-vigorous exercise has been associated with a strong and timely immune response against infections, thus reducing susceptibility to acute respiratory illness, and also protecting from severe COVID-19 outcomes.[2,3] Frequent high-intensity training has also been proposed to enhance vaccine-induced cellular and humoral immunity.[2] However, long-term high-intensity physical activity and stressors associated with elite sports might cause hyperinflammation in some individuals and increase the risk of respiratory illness, and ice hockey players are among those winter sport athletes, who have the highest incidence in that context.[3,4] Proteomic profiling of COVID-19 patients has proven valuable in the discovery of novel biomarkers associated with disease susceptibility, course, complications, and severity,[5] but so far there are no reports of COVID-19 proteomic studies in athletes. Herein, we examined the immune-inflammatory proteome of elite ice hockey players before and after a team-wide COVID-19 outbreak with the omicron BA.1 variant in December 2021.

Serum blood samples and questionnaire data were obtained from 24 players of a Swiss National League ice hockey team 3 months prior to COVID-19 and from the same players within 1–2 weeks after nasal swab PCR-confirmed SARS-CoV-2 infection, and of 20 controls, that are non-ice hockey players after recent recovery from COVID-19. Written informed consent was obtained from all study participants, and the protocol was approved by the responsible ethics committee (Kantonale Ethikkommission Zürich, Ref. 2019–02002). Proximity extension assay (PEA) technology by OLINK was used for targeted proteomic serum analyses of 180 proteins measured in the OLINK immune response and inflammation panels (92 proteins each, 4 overlaps; Figure S1). Immune-inflammatory profiles of ice hockey players were compared at two time points (pre- and post-COVID-19). Additionally, post-COVID-19 profiles of ice hockey players were compared to the post-COVID-19 control group for reference. A detailed description of methods can be found in the Appendix S1.

TABLE 1 Basic characteristics of included ice hockey players and control subjects

	Ice hockey players ($n = 24$)	Post-COVID-19 controls ($n = 20$)
Age (range), sex	26.6 (18–38), male	30.5 (21–43), male
Symptoms (any), *n* (%)	12 (54%*)	19 (95%)
Respiratory symptoms, *n* (%)	8 (36%*)	16 (80%)
Cough	7 (88%)	16 (100%)
Breathlessness	1 (12%)	2 (12%)
Previously SARS-CoV-2-infected[a], *n* (%)	7 (29%)	3 (15%)
Number of vaccinations, *n* (%)		
None	3 (12%)	2 (10%)
At least 1	21 (88%)	18 (90%)
1 (incomplete)	6 (26%)	2 (10%)
2 (basic immunization[b])	7 (29%)	13 (65%)
3 (boostered)	8 (33%)	3 (15%)
Vaccine, *n* (%)		
Moderna (Spikevax®)	18 (86%)	13 (72%)
Pfizer/BioNTech (Comirnaty®)	3 (14%)	4 (22%)
Johnson & Johnson (Janssen®)	0 (0%)	1 (6%)
Asthma, *n* (%)	2 (8%)	4 (21%)
Allergic rhinitis, *n* (%)	3 (4%)	5 (25%)
Regular URTI or fever, *n* (%)	2 (9%**)	1 (5%)
Recurrent herpes labialis, *n* (%)	5 (22%**)	3 (16%)

*$n = 22$; ** $n = 23$.

Abbreviations: COVID-19, coronavirus disease 19; SARS-CoV-2, severe acute respiratory.

[a]Number (*n*) and percentage (%) of subjects that reported a previous SARS-CoV-2 infection since the start of the pandemic, meaning that they got infected twice.

[b]1 vaccination shot of the Janssen® vaccine is considered as basic immunization.

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Ice hockey players and control subjects reported comparable rates of previous SARS-CoV-2 infections, atopic comorbidities (asthma, allergic rhinitis), regularly occurring upper respiratory tract infections (URTI), fever, and recurrent herpes labialis (Table 1). COVID-19 vaccination history did not differ between athletes and controls. Control subjects reported a higher prevalence of symptoms

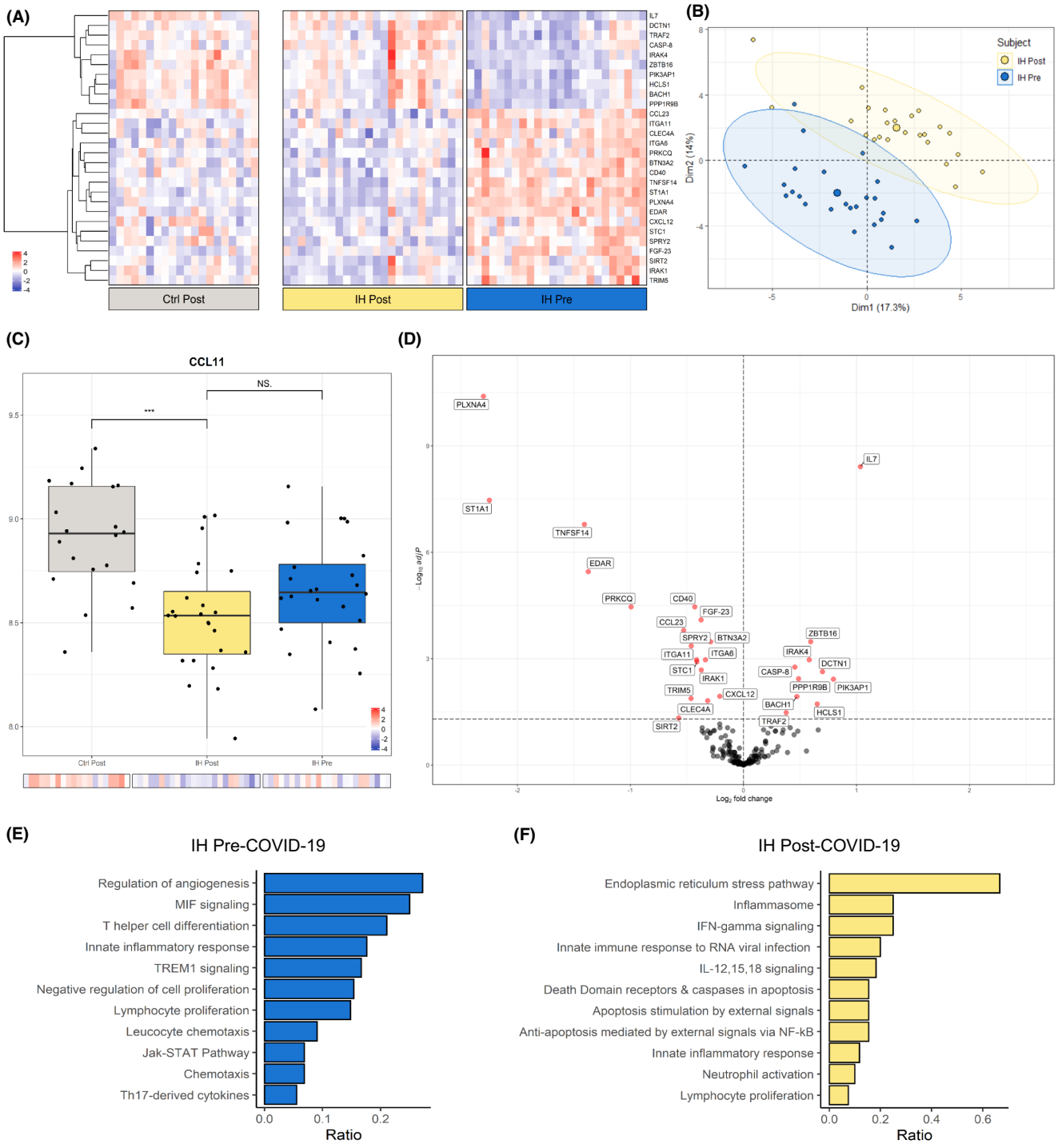

FIGURE 1 Differentially expressed inflammation- and immune response-related proteins pre- and post-COVID-19 in ice hockey players. (A) Heatmap showing differentially expressed proteins among athletes pre- and post-COVID-19, as well as post-COVID controls; (B) principal component analysis (PCA) of ice hockey players pre- and post-COVID-19; (C) scattered boxplot and heatmap of protein CCL11 (***: adj$p$ = .0035); (D) volcano plot of differentially expressed proteins measured by OLINK multiplex assays. Welch 2-sample t-test was used to compare pre- and post-COVID-19 athlete samples (confidence level 0.95). Correction for multiple testing done by Benjamini–Hochberg method. Statistically significant proteins shown in red (adj$p$ < .05); (E, F) ratio of significantly different proteins for different immune response and inflammation process networks to all proteins measured) in pre- (E) and post-COVID-19 athletes (F). Abbreviations: adj$p$, adjusted p value; Ctrl, control group; Dim1, dimension 1; Dim2, dimension 2; IH, ice hockey players; NS, non-significant

in general and respiratory symptoms specifically. Clinical laboratory serum analyses showed no difference between ice hockey players and controls (Table S1).

PEA-based proteomic analyses of serum samples from elite ice hockey players identified 28 differentially expressed proteins involved in immune response and inflammation (Figure 1A,D) with a rather distinct representation of biological process networks (*t*-test results can be found in the Appendix S1). While similar process networks were found to be present at both sampling time points, specifically, lymphocyte proliferation (*CXCL12*, *CD40*, *PRKCQ*, *TNFSF14* pre-COVID-19 and *TRAF2*, *IRAK4*, *CASP-8* post-COVID-19) and innate inflammatory response (*PRKCQ*, *IRAK1* pre-COVID-19 and of *TRAF2*, *IRAK4* post-COVID-19) (Figure 1E,F), the post-COVID-19 profile was uniquely marked by an increase in proteins involved in innate immune response to viral infection, neutrophil activation, IL-12/−15/−18 and IFNγ-signaling (Figure 1F). This last finding therefore may reflect how the immune system responds efficiently to COVID-19, where a timely release of antiviral interferons seems to be essential.[3]

In our principal component analysis (PCA), while we could clearly separate athletes pre- and post-infection (Figure 1B), post-COVID-19 comparison of ice hockey players with post-COVID-19 samples of the control non-ice hockey players group revealed similar proteomic patterns, with only *CCL11* showing a significantly higher expression in the control group (Figure 1C). Of note, levels of *CCL11* have recently been found to be elevated in patients experiencing cognitive symptoms ("brain fog") after mild COVID-19 compared to those without such symptoms.[6] However, we did not assess incidence of post- or long-COVID-19 symptoms in our cohorts.

It could be hypothesized that the higher reported incidence of COVID-19 symptoms in the control group might be linked with a weaker immunological response to either the virus, the vaccination or both, or even due to the fact that more ice hockey players had received a booster shot by the time they got infected (33% vs. 15% of controls). Studies assessing influenza vaccine efficacy in elite athletes found that they had a more pronounced vaccine-induced immune response than healthy controls; however, this has not been investigated for mRNA COVID-19 vaccines thus far.[2] A main limitation of our study is that it cannot be ruled out that the difference in symptom prevalence between the two groups is influenced by selection bias, as all ice hockey players were tested due to the suspected team-wide outbreak (regardless of their clinical presentation) and control subjects might have been more likely to get tested for SARS-CoV-2 if they felt ill.

To conclude, we found that immune-inflammatory proteomic profiles in serum of elite ice hockey players differ significantly pre- and post-COVID-19. The cause for this observation might be multifactorial, for example, direct impact of the recent SARS-CoV-2 infection, seasonal changes, or training-related influences on sampling timepoints. Although proteomic profiles generally did not differ between athletes and control subjects post-COVID-19, the higher incidence of symptomatic disease in the control group warrants follow-up studies to investigate the potential impact of athletic workloads on (SARS-CoV-2) infection susceptibility, disease course, and vaccination response, as well as to identify associated biomarkers.

ACKNOWLEDGEMENTS

This work has been supported by Vontobel-Stiftung and the Center for Data Analysis, Visualization and Simulation (DAViS) funded by the Swiss canton of Grisons. Open access funding provided by Universitat Zurich. [Correction added on 02-July-2022, after first online publication: CSAL funding statement has been added.

KEYWORDS

athletes, COVID-19, immune response, inflammation, proteomics

FUNDING INFORMATION

Center for Data Analysis, Visualization and Stimulation (DAViS); Vontobel-Stiftung

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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