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BRIEF COMMUNICATION

SARS-CoV-2 spike protein functionally interacts with primary human conjunctival epithelial cells to induce a pro-inflammatory response

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After more than 2 years since the emergence of COVID-19, the pandemic is still not under control and many countries struggle with ever-increasing infection rates. Based on reports of COVID-19-associated conjunctivitis [1] along with evidence for ACE2 and TMPRSS2 mRNA and protein expression in the conjunctival epithelium [2], the eye was proposed as a potential viral entry site. Accordingly, Coroneo et al. argued for the eye to play a significant role in transmission and proposed an underappreciation of the recommended eye protection as a potential missing key in fighting the pandemic [3]. Unfortunately, functional evidence on the interaction between SARS-CoV-2 and the ocular surface is scarce. To address this, we generated primary cultures of conjunctival epithelial cells using impression cytology from healthy subjects, treated the cultures with different concentrations of recombinant SARS-CoV-2 spike protein, and monitored the expression of relevant cytokines via qRT-PCR. Ten healthy subjects with a mean age of 29 ± 2 years [7 males (70%); 3 females (30%)] with no apparent or documented ocular pathologies were included. Detailed information on the performed procedures can be found in the Supplementary material. The experimental workflow is depicted in Fig. 1A.

Our data indicate that transfection of conjunctival epithelial cells with SARS-CoV-2 spike protein was sufficient to induce a profound transcriptional response in most of the analyzed genes (Fig. 1B). Expression of pro-inflammatory cytokines such as IL6 and TNFα showed a strong increase after exposure to the SARS-CoV-2 spike protein. GCSF followed a similar trend with a more variable course. In contrast, a long-lasting transcriptional downregulation of anti-inflammatory cytokines like IL4 and IL10 along with the multifunctional cytokine IL2 was observed. MCP1 was upregulated at 48 h after treatment but returned to levels below the control. IP10 showed less consistency between subjects but a general tendency toward upregulation. IL7, a potent immunomodulatory cytokine, showed a highly significant downregulation.

The strong upregulation of IL6 and TNFα is consistent with cytokine profiles found in COVID-19 patients [4] and the significant downregulation of anti-inflammatory cytokines like IL4 and IL10 is typical for viral infections. Furthermore, the observed downregulation of IL2 is of special interest, since IL2/IL2R signaling is a known trigger for $CD8^+$ T-cell activation and decreased IL2/IL2R signaling along with reduced lymphocyte counts [5] was reported in patients with critical courses of COVID-19. The downregulation of IL2 may depict an escape mechanism that the SARS-CoV-2 virus uses to impair the T-cell-mediated host immune response over prolonged periods of infection. In addition, we observed a significant decrease in IL7 expression. IL7 plays a crucial role in lymphocyte proliferation and differentiation and recombinant IL7 has proven to be effective in restoring lymphocyte counts in several viral infections [5]. Likewise, recombinant IL7 was successfully used to treat COVID-19-associated lymphocytopenia [5]. While the results of this study should be interpreted considering its limitations, our findings strongly indicate the potential role of the ocular surface as an entry site for the virus. Further studies are warranted to substantiate these findings and explore the functional consequences in a larger cohort.

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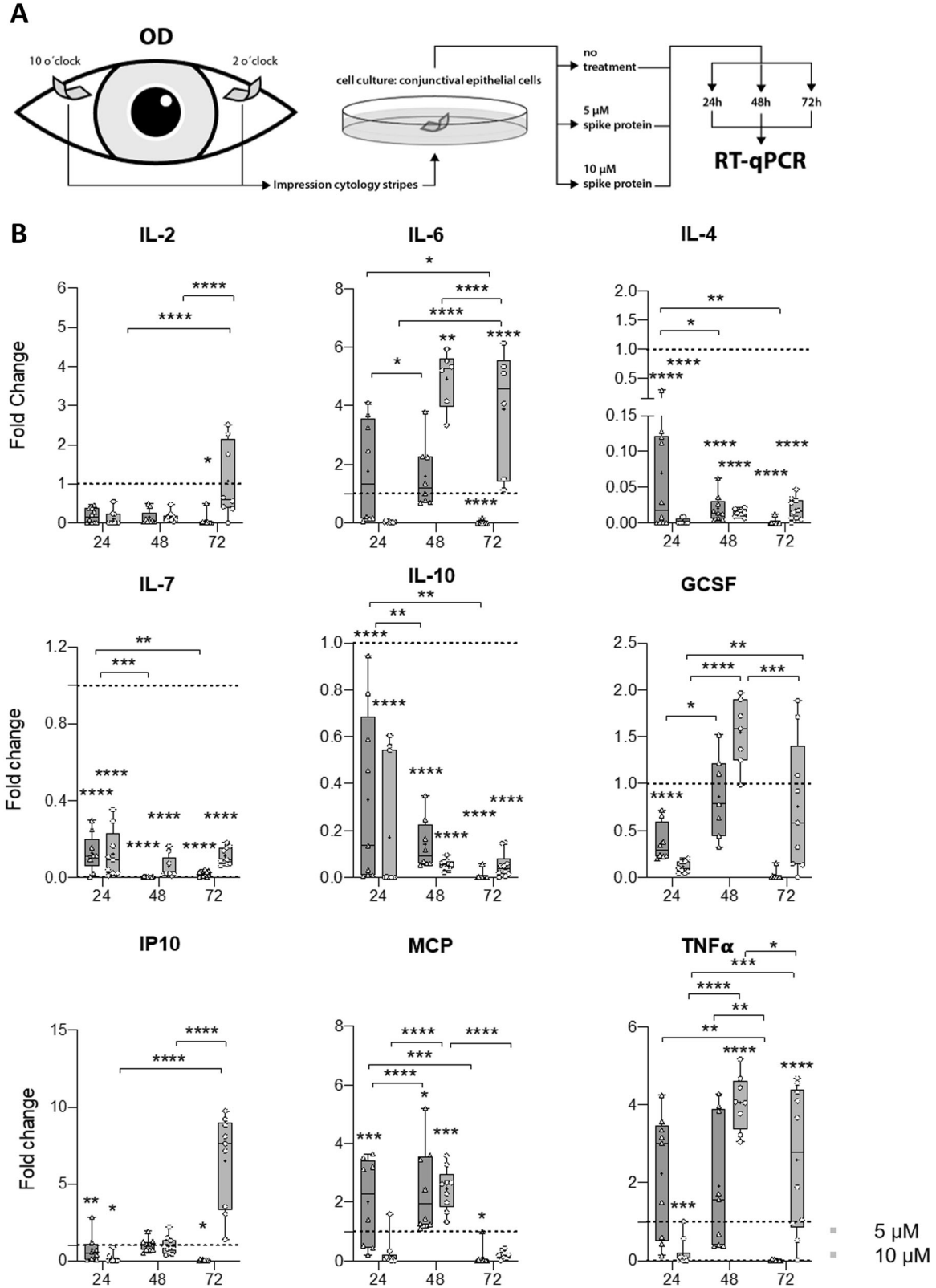

Fig. 1 Experimental workflow and RT-qPCR results. A Illustrative representation of the experimental workflow. Two impression cytology stripes were collected from each patient´s right eye (OD). Stripes were incubated until a sufficient amount of cells were available for treatment with either no, 5 µmol, or 10 µmol SARS-CoV-2 spike protein for 24, 48, and 72 h. Subsequently, RT-qPCR was performed to assess transcriptional response of relevant inflammatory genes. **B** RT-qPCR results. Pro-inflammatory cytokines (IL6, TNFa) were significantly upregulated at most time points, anti-inflammatory cytokines (IL4, IL10) were significantly downregulated as expected for a functional induction of an inflammatory response. Furthermore, IL7 and IL2 were downregulated and MCP and IP10 showed a mixed response with a tendency towards upregulation. Data were normalized to control samples and control values are represented as dashed lines. Significances between two samples are indicated with brackets, significances for comparisons with the control samples are indicated by stars above the respective boxes. One-way ANOVA with Tukey's multiple comparisons test was used to determine statistical significance. *$p > 0.05$, **$p > 0.01$, ***$p > 0.001$, ****$p > 0.0001$.

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AUTHOR CONTRIBUTIONS

Conceptualization: NW, VP, DAM; data analysis: AA, NG-S; investigation: NG-S, NW; resources: PW, AW, JH-W; writing—original draft: DAM; writing—review and editing: NG-S, NW, AA, PW, AW, VP, DAM; visualization: NG-S, VP, DAM; supervision: JH-W, VP, DAM; project administration: NG-S, NW, VP, DAM; funding acquisition: NW, DAM.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41433-022-02066-7.

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