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Photodiagnosis and Photodynamic Therapy 35 (2021) 102457

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Ultraviolet-A light reduces cellular cytokine release from human endotracheal cells infected with Coronavirus

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ARTICLE INFO

Keywords:
Coronavirus-229E
Cytokine storm
Human tracheal epithelial cells
Interleukin-6
Tumor necrosis factor
COVID-19
Ultraviolet light-A
UV-A

ABSTRACT

Background: An important clinical feature of coronavirus disease 2019 (COVID-19) is hypercytokinemia (cytokine storm). We previously showed that narrow band ultraviolet-A (NB-UVA) treatment salvages coronavirus (CoV)-229E-infected human tracheal cells, and that daily endotracheal NB-UVA therapy reduced severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) levels in human subjects, with improved clinical outcomes. Here, we examined NB-UVA effects on cytokine release during CoV-229E infection.

Methods: Primary human tracheal epithelial cells were transfected with CoV-229E, then exposed to 2 mW/cm^2 NB-UVA for 20 minutes every 24h, either 3 or 4 times. Secreted cytokine/chemokine levels were analyzed in supernatants collected from CoV-229E-infected/UVA-exposed cells 24h after the last UVA treatment, and from matched non-infected/UVA-exposed controls, CoV-229E-infected/non-exposed controls, and non-infected/non-exposed (naïve) controls. Metabolic pathway/downstream prediction analyses were also performed.

Results: Pro-inflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF), and chemokines IL-8, monocyte chemoattractant protein-1 (MCP1), and interferon gamma-induced protein 10 (IP-10), were significantly increased in CoV-229E-infected cells, and significantly decreased following NB-UVA treatment. Interferon (IFN)-α2, IFN-γ, and IL-10 were not upregulated in response to CoV-229E. Metabolic pathway predictions indicated hypercytokinemia as the top inflammatory response in CoV-229E-infected cells, whereas the top predicted pathway in CoV-229E-infected/UVA-exposed cells was the recovery stage of severe acute respiratory syndrome.

Conclusions: Human tracheal epithelial cells infected with CoV-229E showed reduced cytokine secretions including IL-6, TNF, IL-8, and MCP-1, following NB-UVA exposure. This reduction of cytokine levels *in vitro*, coupled with previously identified reduced cell death in CoV-229E-infected/UVA-exposed cells, suggests that determining UVA effects on cytokine storm in human SARS-Co-V2 patients is warranted.

1. Introduction

The recent coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has taken a tragic toll globally [1]. Those with severe symptoms primarily present with acute respiratory distress. The main mechanism by which SARS-CoV-2 initially induces respiratory distress is through infection of ciliated epithelial cells primarily located in the nasal passage, trachea, and larger airways [2]. Progression of infection leads to release of inflammatory cytokines, epithelial cell death with ensuing exudates and thick mucus plugs [3], acute respiratory distress syndrome (ARDS) [4], and predisposition to secondary infections such as ventilator-associated pneumonia [5]. The term “cytokine storm” [6] describes surging levels of cytokines produced by airway epithelial cells in response to SARS-CoV-2. These cytokines include interleukin (IL)-6, tumor necrosis factor (TNF), interferon gamma-induced protein 10 (IP-10, also known as C-X-C motif chemokine ligand 10 (CXCL10)), and macrophage inflammatory protein (MIP)-1α and 1β [7-9]. Excessive elevation of these

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https://doi.org/10.1016/j.pdpdt.2021.102457
Received 16 June 2021; Received in revised form 12 July 2021; Accepted 22 July 2021
Available online 24 July 2021

cytokines and hyperactivation of immune cells is associated with poor patient outcomes and death in COVID-19 patients [10]. Given the prevalence of cytokine storm in COVID-19 patients, several anti-inflammatory agents are used to treat moderate-to-severe infection, such as tocilizumab, glucocorticoids, and baricitinib [11].

Recently, we have shown that narrow band ultraviolet-A (NB-UVA) light can be used to treat coronavirus (CoV)-229E infected human tracheal epithelial cells [12] and human patients with critical SARS-CoV-2 infection under specific and monitored settings [13]. CoV-229E is a positive-sense single-stranded RNA coronavirus mainly associated with mild upper respiratory infections, whereas SARS-CoV-2 and middle east respiratory syndrome (MERS)-CoV are associated with severe lower respiratory tract infection [14, 15]. *In vitro*, CoV-229E also generates a proinflammatory response, which primarily occurs after the first 24 hours of infection [16, 17]. This inflammatory response includes increases in IL-1β, IL-6, IL-8, IP-10, IFN-β, many of which do not peak until 96 hours after infection [17]. As CoV-229E is a safer virus to study (biosafety level (BSL)-2), we have used CoV-229E as a model to explore the mechanisms by which NB-UVA light can reduce inflammation in tracheal epithelial cells, as observed in human COVID-19 patients who underwent endotracheal treatment with NB-UVA light [13]. We previously showed that in primary human ciliated tracheal epithelial cells infected with CoV-229E, exposure to NB-UVA light improved cell viability, salvaging them from the cell death seen in CoV-229E infected cells that were not exposed to NB-UVA [12]. Interestingly, NB-UVA treatment appeared to be associated with an increase in levels of mitochondrial anti-viral signaling protein (MAVS) [12, 18]. Although MAVS has been shown not to increase in the first 24 hours of SARS-CoV-2 infection [19], SARS-CoV-2 is known to antagonize MAVS, thus impairing MAVS-mediated innate antiviral responses to the virus [20]. Moreover, we recently found that exposure to UVA light significantly increased MAVS levels not only in cells directly exposed to NB-UVA, but also across monolayer cells that had no direct NB-UVA exposure, indicating that MAVS activation was likely transmitted via cell-to-cell signaling [18]. This suggested that local exposure to NB-UVA could have more distant consequences.

In a first-in-human study, application of NB-UVA inside the trachea of ventilated COVID-19 patients resulted in a $>3\ \log_{10}$ mean reduction of

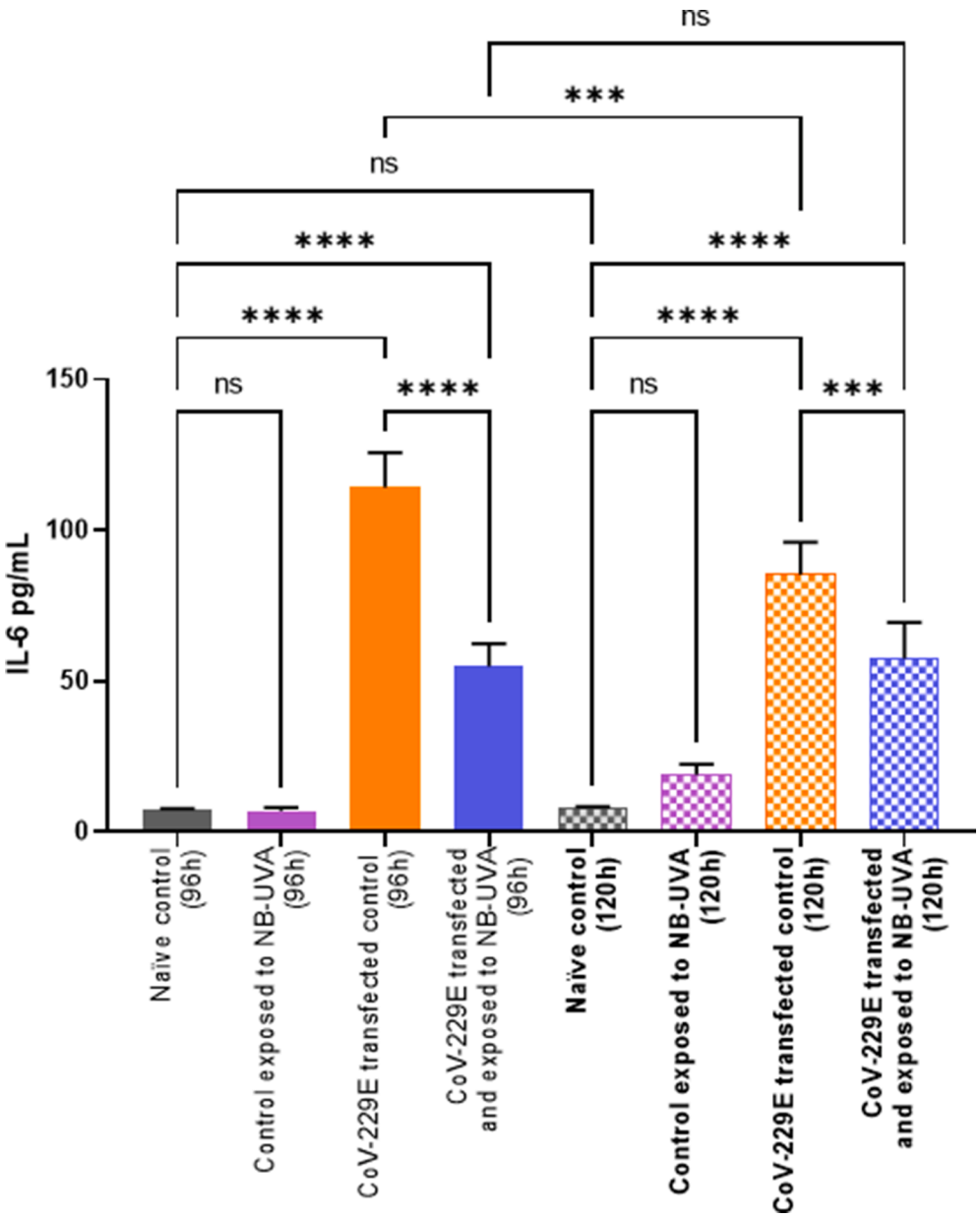

Fig. 1. Normalized IL-6 levels in the supernatants of CoV-229E transfected HTEpC treated 3 times (96h treated group) or 4 times (120h treated group) with 2 mW/cm^2 of NB-UVA for 20 minutes, and in the supernatants of CoV-229E transfected HTEpC not exposed to NB-UVA (96h and 120h CoV-229E transfected controls), noninfected HTEpC exposed 3x and 4x to NB-UVA (96h and 120h UVA-treated controls), and noninfected HTEpC not exposed to NB-UVA (96h and 120h naïve controls). **** P<0.0001, *** P<0.001, ** P<0.01, * P<0.05. Mean ± standard deviation of cell viability determined by Trypan Blue 0.4% at 96h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 95% (±3%), noninfected HTEpC exposed to NB-UVA – 97% (±1%), CoV-229E transfected HTEpC exposed to NB-UVA – 94% (±3%), CoV-229E transfected HTEpC not exposed to NB-UVA – 86% (±3.5%). Cell viability at 120h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 92% (±1%), noninfected HTEpC exposed to NB-UVA – 96.6% (±3.5%), CoV-229E transfected HTEpC exposed to NB-UVA – 95% (±2.5%), CoV-229E transfected HTEpC not exposed to NB-UVA – 70% (±4.4%).

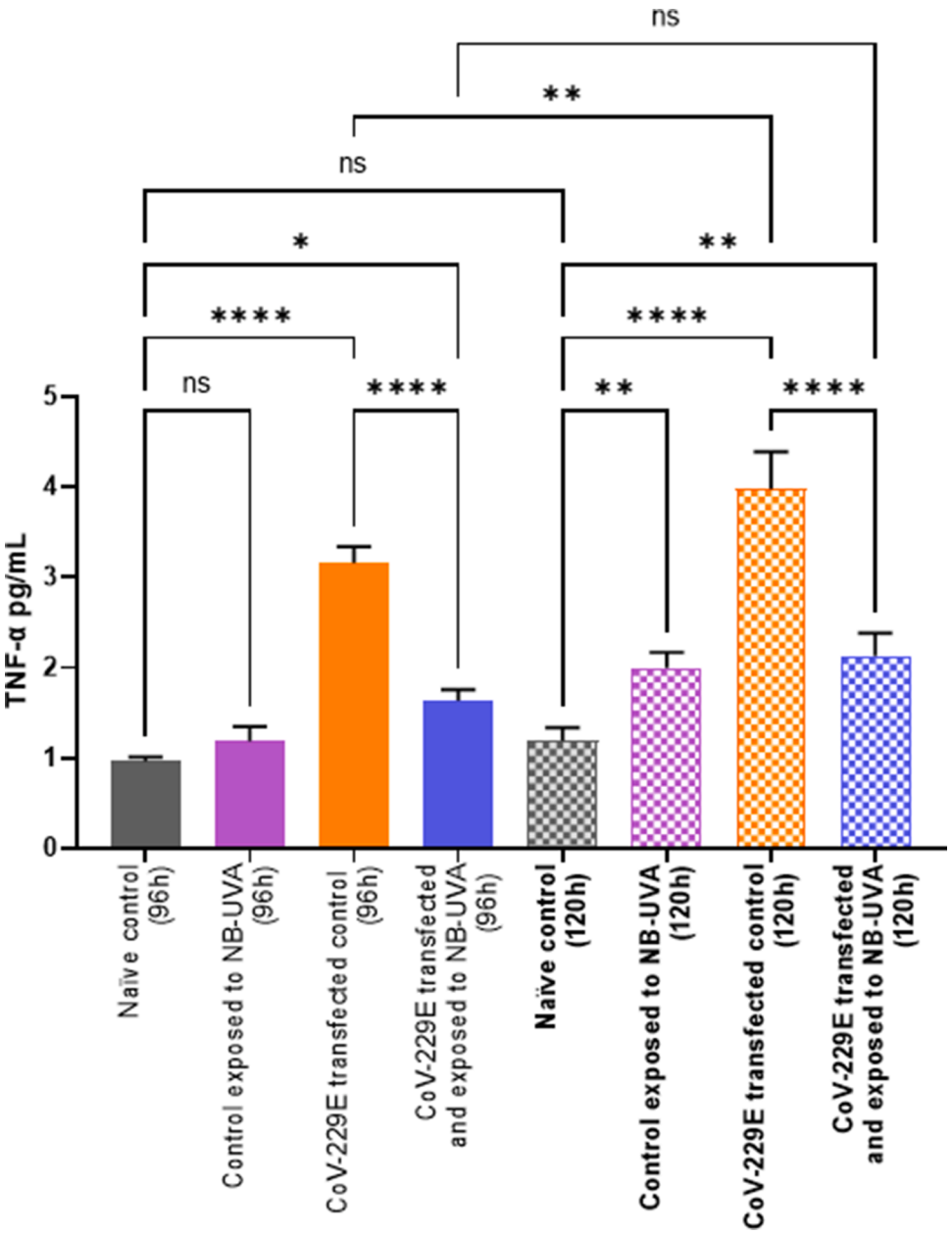

Fig. 2. Normalized TNF-α levels in the supernatants of CoV-229E transfected HTEpC treated 3 times (96h treated group) or 4 times (120h treated group) with 2 mW/cm^2 of NB-UVA for 20 minutes, and in the supernatants of CoV-229E transfected HTEpC not exposed to NB-UVA (96h and 120h CoV-229E transfected controls), noninfected HTEpC exposed to NB-UVA (96h and 120h UVA-treated controls), and noninfected HTEpC not exposed to NB-UVA (96h and 120h naïve controls). **** P<0.0001, *** P<0.001, ** P<0.01, * P<0.05. Mean ± standard deviation of cell viability determined by Trypan Blue 0.4% at 96h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 95% (±3%), noninfected HTEpC exposed to NB-UVA – 97% (±1%), CoV-229E transfected HTEpC exposed to NB-UVA – 94% (±3%), CoV-229E transfected HTEpC not exposed to NB-UVA – 86% (±3.5%). Cell viability at 120h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 92% (±1%), noninfected HTEpC exposed to NB-UVA – 96.6% (±3.5%), CoV-229E transfected HTEpC exposed to NB-UVA – 95% (±2.5%), CoV-229E transfected HTEpC not exposed to NB-UVA – 70% (±4.4%).

SARS-CoV-2 levels in respiratory samples, and the degree of this reduction after NB-UVA therapy correlated with a reduction in serum C-reactive protein and 30-day clinical improvements in treated patients [13]. Although NB-UVA treatment appears promising, the effects of NB-UVA on the cytokine response initiated by human tracheal cells remains to be determined. In this study, we aimed to examine the cytokine response in human tracheal epithelial cells infected with coronavirus, and to assess whether controlled application of NB-UVA light to these cells influences cytokine responses.

2. Materials and methods

2.1. Preparation of CoV-229E

Human CoV-229E (ATCC VR-740, ATCC) was overlain onto confluent MRC-5 human lung fibroblasts. CoV-229E is considered lytic [21]. Once cells exhibited ~50% cytopathic effect, cells were trypsinized and the cell/media suspension was collected. The cell/media mixture underwent one rapid freeze/thaw cycle and was centrifuged at 1000x g for 10min to clarify the media. The virus-containing supernatants were used in subsequent experiments.

NB-UVA exposure of human primary tracheal epithelial cells transfected with CoV-229E

Primary human tracheal epithelial cells isolated from the surface epithelium of human trachea (HTEpC, Lot 446Z036.8, Male, age 50, Caucasian, PromoCell GmbH, Heidelberg, Germany) were cultured at 37°C (5% CO_2) in 60 × 15mm standard tissue culture dishes (cat. 351007, Corning, NY, USA) with Airway Epithelial Cell Growth Medium (cat. C-21060, PromoCell) prepared with SupplementMix (cat. C-39165, PromoCell) and Gibco antibiotic-antimycotic solution (cat. 15240096, ThermoFisher Scientific, MA, USA). When HTEpC reached 30-40% confluency, 50 μL of the CoV-229E-containing supernatants were added to the cell medium, and the cells were transfected at 37°C (5% CO_2) for 24h. HTEpC were then washed 3 times with sterile 1xPBS pH 7.4 (cat. 10010072, ThermoFisher), and 5 mL fresh media was added to the cell cultures. HTEpC not transfected with CoV-229E were used as naïve

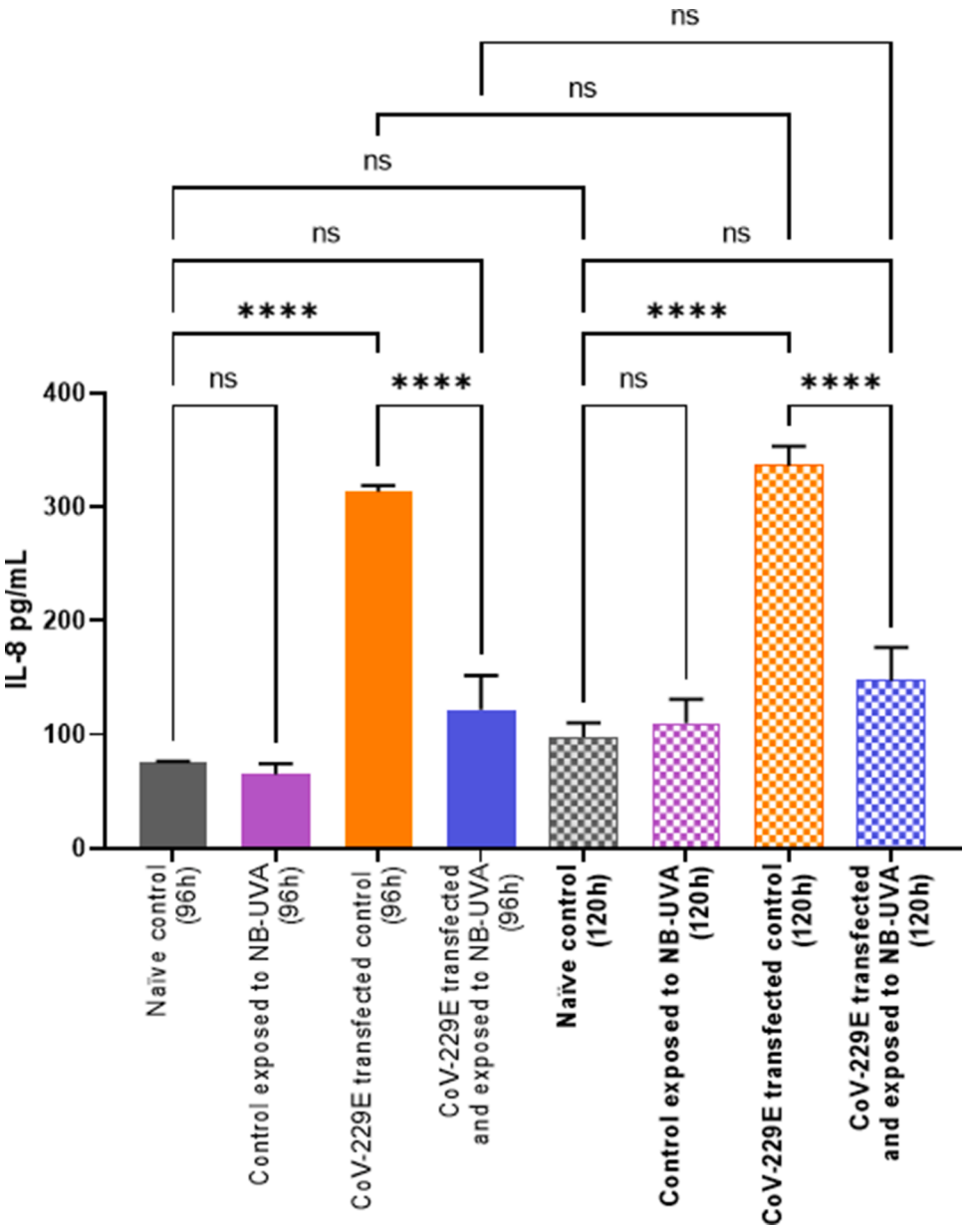

Fig. 3. Normalized IL-8 levels in the supernatants of CoV-229E transfected HTEpC treated 3 times (96h treated group) or 4 times (120h treated group) with 2 mW/cm^2 of NB-UVA for 20 minutes, and in the supernatants of 96h and 120h CoV-229E transfected controls, 96h and 120h UVA-treated controls, and 96h and 120h naïve controls. **** P<0.0001, *** P<0.001, ** P<0.01, * P<0.05. Mean ± standard deviation of cell viability determined by Trypan Blue 0.4% at 96h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 95% (±3%), noninfected HTEpC exposed to NB-UVA – 97% (±1%), CoV-229E transfected HTEpC exposed to NB-UVA – 94% (±3%), CoV-229E transfected HTEpC not exposed to NB-UVA – 86% (±3.5%). Cell viability at 120h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 92% (±1%), noninfected HTEpC exposed to NB-UVA – 96.6% (±3.5%), CoV-229E transfected HTEpC exposed to NB-UVA – 95% (±2.5%), CoV-229E transfected HTEpC not exposed to NB-UVA – 70% (±4.4%).

controls.

For NB-UVA experiments, an array of light-emitting diodes (LEDs) (Seoul Viosys, Gyeonggi-Do, South Korea; peak wavelength 343±3nm, with full width at half maximum of 5nm) mounted on an aluminum heatsink was used. Wavelength and intensity were confirmed by spectrometry (Flame UV-VIS, Ocean Optics, FL) and UV meters (SDL470 and UV510 UV, Extech, NH). The distance from the UVA source to the target tissue was 4 cm for all experiments. HTEpC transfected with CoV-229E were exposed to 2 mW/cm^2 NB-UVA for 20 minutes every 24h, either 3 times (n=3, 3x treatment group) or 4 times (n=3, 4x treatment group). Transfected HTEpC that were not exposed to NB-UVA were used as CoV-229E infected controls (n=6), and noninfected, non-exposed HTEpC were used as naïve controls (n=6). HTEpC that were not infected with CoV-229E but were exposed to NB-UVA 3 times (n=3) or 4 times (n=3) were used as UVA-treated controls. Supernatants were obtained from CoV-229E transfected HTEpC 24h after the last treatment with NB-UVA (i.e. at 96h for HTEpC that underwent 3 UVA treatments and at 120h for HTEpC that underwent 4 UVA treatments). Supernatants were also obtained from CoV-229E infected controls and naïve controls at matching timepoints (i.e. at 96h to match cells that underwent 3 UVA treatments and at 120h to match cells that underwent 4 UVA treatments).

NB-UVA effects on pro-inflammatory cytokines and chemokines in CoV-229E transfected tracheal cells

Secreted pro- and anti-inflammatory cytokines/chemokines were analyzed in supernatants from all treated and control groups. Interferon alpha (IFN-α), IFN gamma (IFN-γ), IL-10, IL-1β, IL-6, IL-8, IP-10, MIP-1β, monokine induced by gamma (MIG, also known as CXCL9), monocyte chemoattractant protein-1 (MCP1) and TNF-α were quantitated using a Milliplex bead-based assay (cat. HCYTOMAG-60K-09, MilliporeSigma, Burlington, MA, USA) on a FLEXMAP 3D® (Luminex Corporation, Austin, TX, USA), according to the manufacturer's instructions. Absolute numbers of live cells were used normalize levels of cytokines. Live cell counts were obtained using an automated cell counter (Biorad T20, Hercules, CA) after staining with Trypan Blue 0.4% (1:1) (Gibco, Waltham, MA).

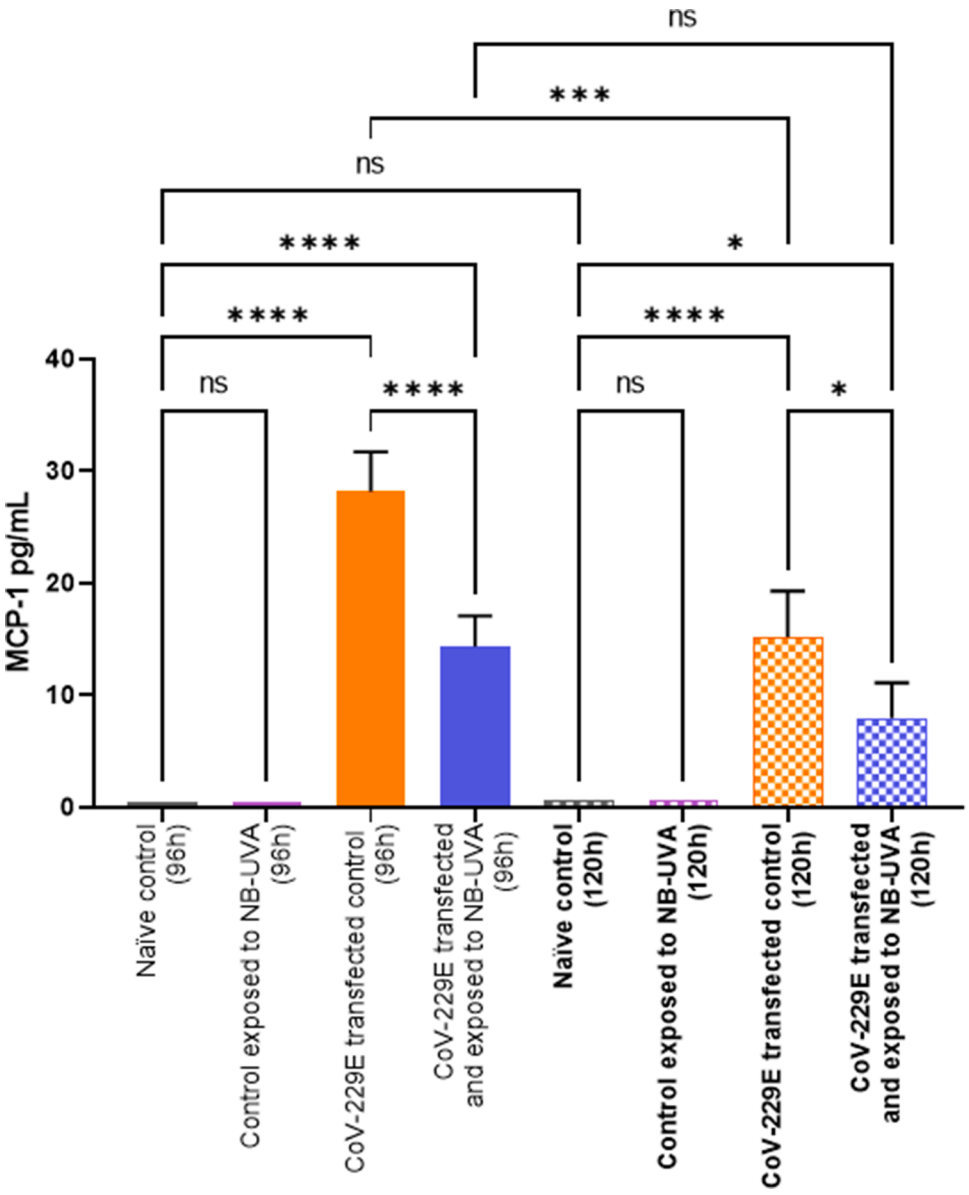

Fig. 4. Normalized MCP-1 levels in the supernatants of CoV-229E transfected HTEpC treated 3 times (96h treated group) or 4 times (120h treated group) with 2 mW/cm^2 of NB-UVA for 20 minutes, and in the supernatants of 96h and 120h CoV-229E transfected controls, 96h and 120h UVA-treated controls, and 96h and 120h naïve controls. **** P<0.0001, *** P<0.001, ** P<0.01, * P<0.05. Mean ± standard deviation of cell viability determined by Trypan Blue 0.4% at 96h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 95% (±3%), noninfected HTEpC exposed to NB-UVA – 97% (±1%), CoV-229E transfected HTEpC exposed to NB-UVA – 94% (±3%), CoV-229E transfected HTEpC not exposed to NB-UVA – 86% (±3.5%). Cell viability at 120h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 92% (±1%), noninfected HTEpC exposed to NB-UVA – 96.6% (±3.5%), CoV-229E transfected HTEpC exposed to NB-UVA – 95% (±2.5%), CoV-229E transfected HTEpC not exposed to NB-UVA – 70% (±4.4%).

2.2. Statistical analysis

Graph construction and statistical analysis were performed with GraphPad Prism V. 9.1.0 (GraphPad Software, CA, USA). Absolute quantities of cytokines and chemokines from supernatants were normalized using the average number of total live cells obtained from each group. After normalization, cytokine and chemokine quantities were compared between groups, applying a non-paired parametrical test with post-test correction. Significance was set at $p < 0.05$.

Metabolic pathway/downstream prediction analyses were carried out using Ingenuity Pathway Analysis (IPA) software (Qiagen, Valencia, CA, USA). Core analyses were performed considering only experimentally observed molecules and/or relationships.

3. Results

Narrow band UVA (NB-UVA) reduces pro-inflammatory responses in CoV-229E transfected tracheal cells

Normalized secreted levels of IL-6 were not significantly different in HTEpC that were exposed to NB-UVA without prior CoV-229E transfection (UVA-treated controls) when compared to naïve controls at any timepoint (Fig. 1). IL-6 levels in CoV-229E transfected HTEpC that were not exposed to NB-UVA (CoV-229E transfected controls) were 15.81-fold higher than levels in naïve controls at 96h (mean=114.2±11.56 pg/mL vs. 7.22±0.46 pg/mL respectively, P<0.0001, Fig. 1). Similar results were also obtained at 120h (fold change (FC)=11.15, mean=85.42±10.56 pg/mL vs. 7.66±0.61 pg/mL, P<0.0001, Fig. 1). In CoV-229E-transfected HTEpC that underwent 3 NB-UVA treatments (i.e. the 96h treated group), levels of IL-6 were decreased 2.08-fold when compared to matched CoV-229E transfected controls (mean=54.80±7.53 pg/mL vs. 114.2±11.56 pg/mL respectively, P<0.0001, Fig. 1). Similar results were obtained for CoV-229E transfected HTEpC that underwent 4 NB-UVA treatments (i.e. the 120h treated group) when compared to matched CoV-229E transfected controls (FC=-1.48, mean=57.34±12.04 pg/mL vs. 85.42±10.56 pg/mL respectively, P=0.0005, Fig. 1).

Normalized secreted levels of TNF-α at 96h were not significantly different in HTEpC that were exposed to NB-UVA without prior CoV-229E transfection (UVA-treated controls) when compared to naïve controls, but increased modestly at 120h, i.e. after 4 NB-UVA treatments (Fig. 2). TNF-α levels in in CoV-229E transfected controls were 3.28-fold higher than levels in naïve controls at 96h (mean=3.16±0.18 pg/mL vs.

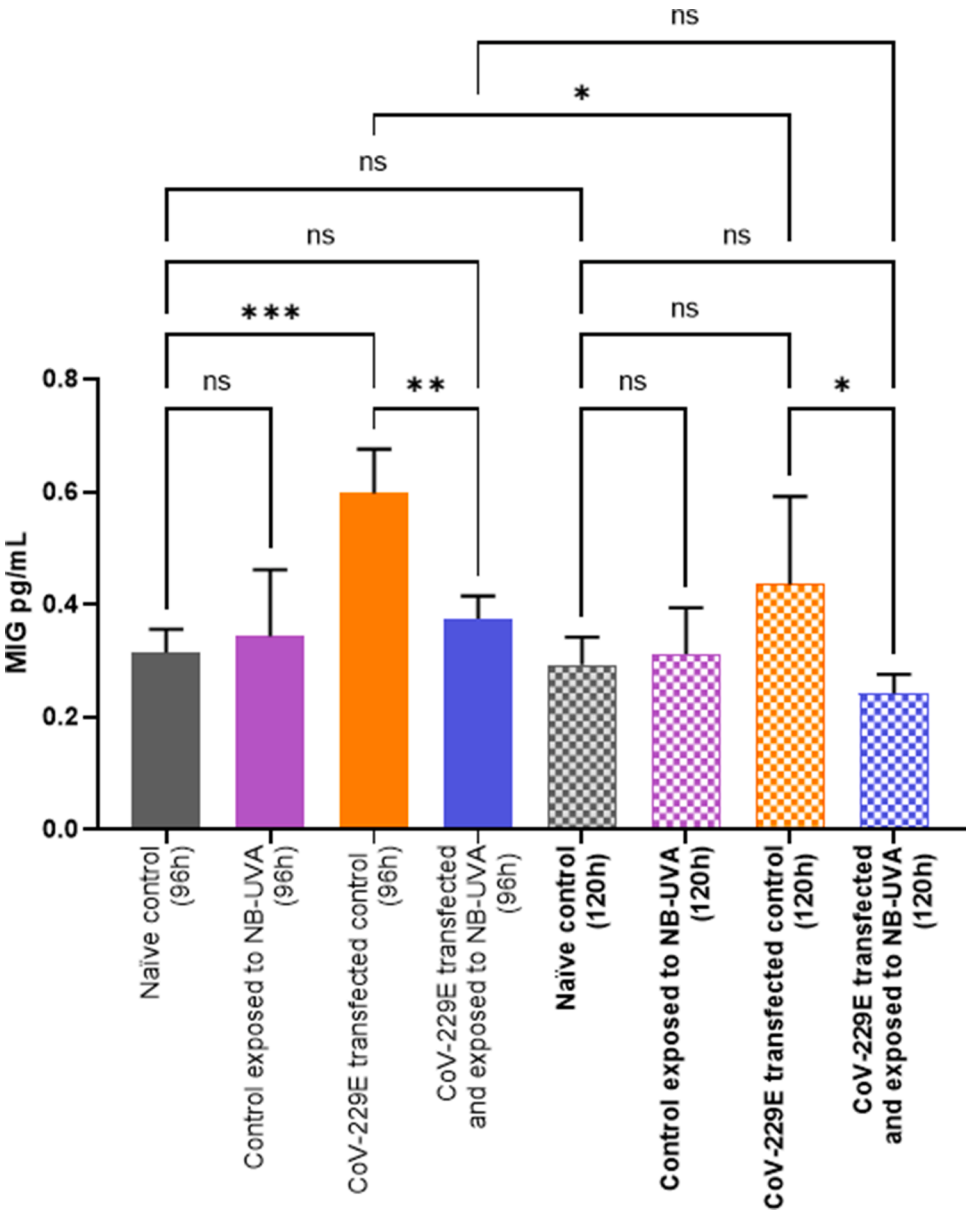

Fig. 5. Normalized MIG levels in the supernatants of CoV-229E transfected HTEpC treated 3 times (96h treated group) or 4 times (120h treated group) with 2 mW/cm^2 of NB-UVA for 20 minutes, and in the supernatants of 96h and 120h CoV-229E transfected controls, 96h and 120h UVA-treated controls, and 96h and 120h naïve controls. **** P<0.0001, *** P<0.001, ** P<0.01, * P<0.05. Mean ± standard deviation of cell viability determined by Trypan Blue 0.4% at 96h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 95% (±3%), noninfected HTEpC exposed to NB-UVA – 97% (±1%), CoV-229E transfected HTEpC exposed to NB-UVA – 94% (±3%), CoV-229E transfected HTEpC not exposed to NB-UVA – 86% (±3.5%). Cell viability at 120h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 92% (±1%), noninfected HTEpC exposed to NB-UVA – 96.6% (±3.5%), CoV-229E transfected HTEpC exposed to NB-UVA – 95% (±2.5%), CoV-229E transfected HTEpC not exposed to NB-UVA – 70% (±4.4%).

0.96±0.04 pg/mL respectively, P<0.0001, Fig. 2). Although similar results were observed at 120h for CoV-229E transfected and naïve control groups (FC=3.36, mean=3.98±0.41 pg/mL vs. 1.18±0.15 pg/mL, P<0.0001, Fig. 2), TNF-α levels were increased even further in CoV-229E transfected controls at 120h when compared to 96h (FC=1.26, P=0.0041, Fig. 2). In CoV-229E transfected HTEpC that underwent 3 NB-UVA treatments (96h treatment group), TNFα levels were decreased 1.93-fold when compared to matched CoV-229E transfected controls (mean=1.63±0.12 pg/mL vs. 3.16±0.18 pg/mL respectively, P<0.0001, Fig. 2). After 4 NB-UVA treatments (120h treatment group), TNF-α levels were further reduced, resulting in a 1.87-fold reduction when compared to CoV-229E transfected controls at 120h (mean=2.13±0.25 pg/mL vs. 3.98±0.41 pg/mL, P<0.0001, Fig. 2).

Secreted levels of two chemokines (i.e. IL-8 and MCP-1) were also analyzed. Normalized secreted levels of IL-8 were not significantly different in UVA-treated controls when compared to naïve controls at any timepoint. IL-8 levels in CoV-229E infected controls were 4.16-fold higher than levels in naïve controls at 96h (mean=313.57±4.94 pg/mL vs. 75.29±1.25 pg/mL respectively, P<0.0001, Fig. 3). Similar results were obtained at 120h (FC=3.43, Mean=336.26±17.06 pg/mL vs. 97.91±12.42 pg/mL, P<0.0001, Fig. 3). In CoV-229E transfected HTEpC that underwent 3 NB-UVA treatments (96h treated group), levels of IL-8 were decreased 2.57-fold when compared to CoV-229E transfected controls at 96h (mean=122.00 ±29.86 pg/mL vs. 313.57±4.94 pg/mL respectively, P<0.0001, Fig. 3). Similar results were obtained after 4 NB-UVA treatments when compared to CoV-229E transfected controls at 120h (FC=-2.28, mean=147.29±29.43 pg/mL vs. 336.26±17.06 pg/mL, P<0.0001, Fig. 3). Interestingly, levels of IL-8 were not significantly different in CoV-229E transfected HTEpC that underwent 3 and 4 NB-UVA treatments when compared to naïve controls (P>0.05, Fig. 3).

Levels of the chemokine MCP-1 were below the detection limit in naïve controls (< 3.2 pg/mL), so for statistical analysis, levels in this group were adjusted to half of the detection limit and then normalized as previously described. Normalized secreted levels of MCP-1 were not significantly different in UVA-treated controls when compared to naïve controls at any timepoint. MCP-1 levels in CoV-229E transfected controls were 48.32-fold higher than levels in naïve controls at 96h (mean=28.16±3.56 pg/mL vs. 0.58±0 pg/mL respectively, P<0.0001, Fig. 4). Similar results were obtained at 120h (FC=29.11,

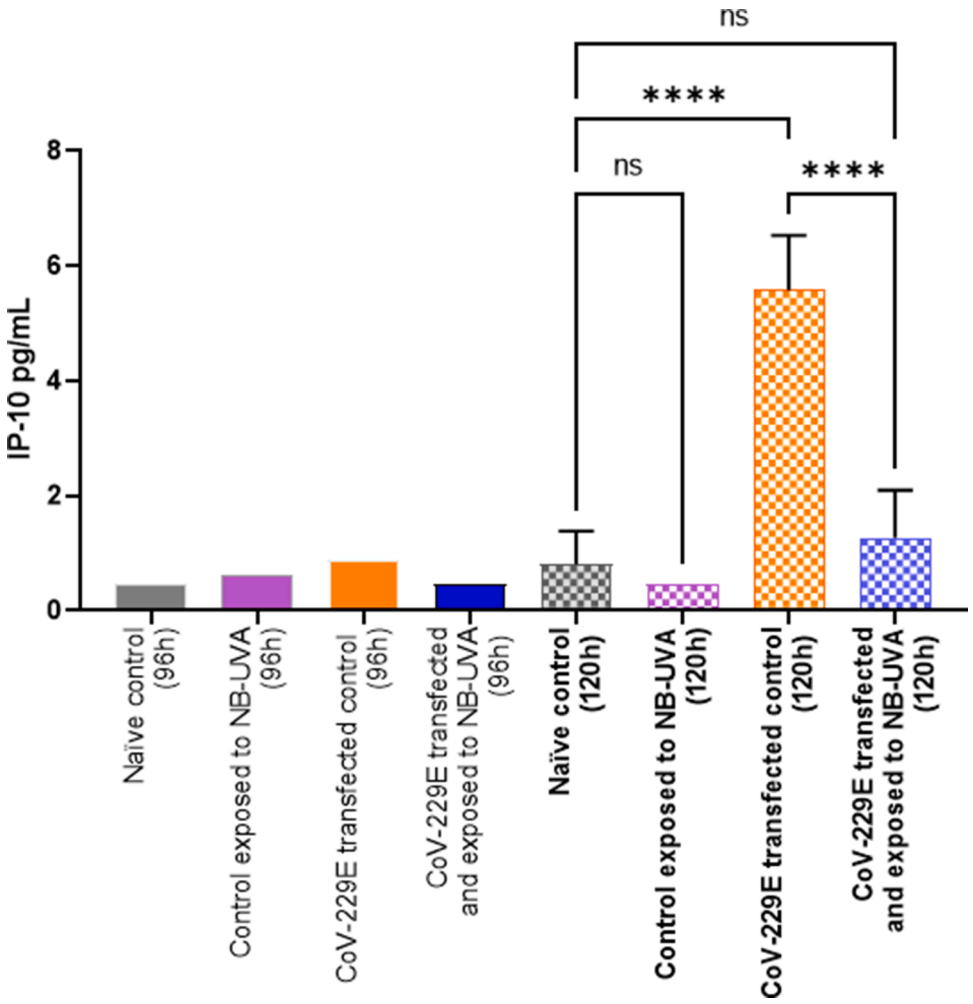

Fig. 6. Normalized IP-10 levels in the supernatants of CoV-229E transfected HTEpC treated 3 times (96h treated group) or 4 times (120h treated group) with 2 mW/cm^2 of NB-UVA for 20 minutes, and in the supernatants of 96h and 120h CoV-229E transfected controls, 96h and 120h UVA-treated controls, and 96h and 120h naïve controls. **** P<0.0001, *** P<0.001, ** P<0.01, * P<0.05. Mean ± standard deviation of cell viability determined by Trypan Blue 0.4% at 96h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 95% (±3%), noninfected HTEpC exposed to NB-UVA – 97% (±1%), CoV-229E transfected HTEpC exposed to NB-UVA – 94% (±3%), CoV-229E transfected HTEpC not exposed to NB-UVA – 86% (±3.5%). Cell viability at 120h (% of live cells): noninfected HTEpC not exposed to NB-UVA – 92% (±1%), noninfected HTEpC exposed to NB-UVA – 96.6% (±3.5%), CoV-229E transfected HTEpC exposed to NB-UVA – 95% (±2.5%), CoV-229E transfected HTEpC not exposed to NB-UVA – 70% (±4.4%).

mean=15.21±4.10 pg/mL vs. 0.52±0 pg/mL, P<0.0001, Fig. 4). In CoV-229E-transfected HTEpC that underwent 3 NB-UVA treatments (96h treated group), levels of MCP-1 decreased 1.96-fold when compared to CoV-229E infected controls at 96h (mean=14.34±2.76 pg/mL vs. 28.16±3.56 pg/mL respectively, P<0.0001, Fig. 4). Similarly, levels of MCP-1 in the 4x treated group were decreased 1.91-fold when compared to CoV-229E infected controls at 120h (mean= 7.97±3.17 pg/mL vs. 15.21±4.10 pg/mL, P=0.035, Fig. 4).

Normalized secreted levels of MIG were not significantly different in UVA-treated controls when compared to naïve controls at any timepoint. MIG levels in CoV-229E transfected controls were 1.9-fold higher than levels in naïve controls at 96h only (mean=0.59±0.08 pg/mL vs. 0.31±0.04 pg/mL respectively, P=0.0009, Fig. 5). In CoV-229E-transfected HTEpC that underwent 3 NB-UVA treatments (96h treated group), levels of MIG decreased 1.59-fold when compared to CoV-229E transfected controls at 96h (mean=0.37±0.04 pg/mL vs. 0.59±0.08 pg/mL respectively, P=0.0056, Fig. 5). In addition, MIG levels were decreased 1.55-fold in the 4x treated group when compared to CoV-229E transfected controls at 120h (mean= 0.24±0.03 pg/mL vs. 0.43±0.16 pg/mL, P=0.0136, Fig. 5).

Levels of IP-10 were not detectable (below the detection limit of the kit) at 96h in any of the groups analyzed. At 120h, normalized secreted levels of IP-10 were not significantly different in UVA-treated controls when compared to naïve controls (P=0.99, Fig. 6). IP-10 levels in CoV-229E transfected controls were 6.92-fold higher than levels in naïve controls at 120h (mean=5.58±0.95 pg/mL vs. 0.80±0.58 pg/mL respectively, P<0.0001, Fig. 6). IP-10 levels in the 4x treated group were significantly lower than levels detected in CoV-229E transfected controls at 120h (FC=-4.40, mean=1.27±0.83 pg/mL vs. 5.58±0.95 pg/mL, P<0.0001, Fig. 6).

Normalized levels of IL-1β were not different between any of the groups tested (data not shown). In addition, CoV-229E infection did not appear to induce upregulation of IFN-α2, IFN-γ, or IL-10, as these cytokines were not detectable in any of the samples analyzed.

Narrow band UVA (NB-UVA) appears to ameliorate pro-inflammatory cytokine/chemokine storm in CoV-229E transfected tracheal cells

Metabolic pathway predictions built based on the pattern of differences between cytokine and chemokine levels in CoV-229E transfected controls and naïve controls at the 120h timepoint revealed an immune response often detected in hypercytokinemia (cytokine storm). This was the top inflammatory response function annotated as predicted to be activated in CoV-229E transfected controls (z-score = 2, P=2.74E-23, Fig. 7A,B). Inflammatory response, infectious diseases, and organismal injury and abnormalities were amongst the top 5 diseases and disorders predicted during analysis (P<0.05, Fig. 7C). Interestingly, ribonuclease A (RNASE1), which has previously been proposed to have antiviral activity [22], was within the top 5 upstream regulators of the immune pathways predicted in CoV-229E transfected controls (P=2.15E-18, Fig. 7D).

When CoV-229E transfected HTEpC were treated 4 times with NB-UVA (120h), the prediction scores changed (Fig. 8 A-C). The top function annotated (infectious disease) was the recovery stage of severe acute respiratory syndrome (P=4.83E-12), but the activation/inhibition state of this function could not be predicted. Hypercytokinemia was only the third inflammatory function annotated during predictions

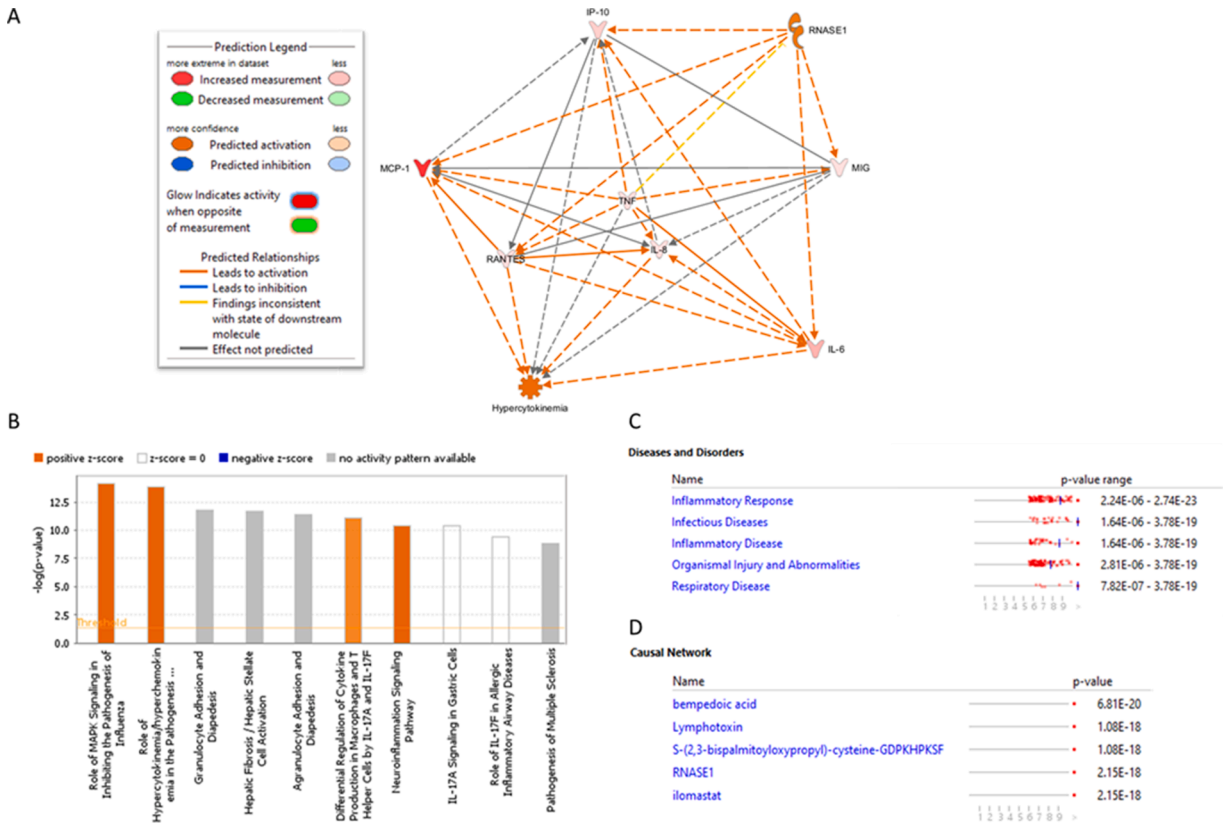

Fig. 7. A. Prediction network showing the interactions between cytokines/chemokines and activation of the hypercytokinemia (cytokine storm) pathway in CoV-229E transfected controls at 120h. **B.** Top 10 canonical pathways predicted to be associated with the effects of CoV-229E, based on cytokine/chemokine patterns obtained *in vitro*. **C.** Top 5 diseases and disorders predicted to be associated with the effects of CoV-229E, based on cytokine/chemokine patterns obtained *in vitro*. **D.** Top 5 upstream regulators associated with the cytokine/chemokine patterns obtained in CoV-229E transfected controls.

(P=3.28E-10, Fig. 8B), but again the activation/inhibition state of this function could no longer be calculated. In contrast to predictions built with CoV-229E transfected controls, RNASE1 was no longer amongst the top 5 upstream regulators of the immune pathways predicted in CoV-229E transfected HTEpC treated with NB-UVA (Fig. 8D).

4. Discussion

In this study, we show that transfection with CoV-229E results in excessive cytokine production in human ciliated tracheal epithelial cells *in vitro*, and that these cytokines are significantly ameliorated following repeated exposure to specific and monitored NB-UVA light therapy. We also show that this effect can be identified after three daily 20-minute treatments with NB-UVA, but appears to be further enhanced after four NB-UVA treatments.

External UVA light therapy is effective in patients with atopic dermatitis [23] and other skin disorders [24], and is FDA-approved for the treatment of conditions such as psoriasis, eczema and skin lymphoma [25]. To explore the potential of internal UVA light therapy to treat microbial infections, we recently tested NB-UVA efficacy against a variety of bacterial, fungal and viral pathogens *in vitro*, including coronavirus-229E [12]. Importantly, we found that human ciliated tracheal epithelial cells (HTEpC) that were infected with CoV-229E and then treated with NB-UVA light exhibited increased survival compared to untreated controls [12]. Moreover, the increased survival of NB-UVA treated infected cells was associated with a decrease in CoV-229E viral load following UVA exposure. We hypothesized that such an effect might be driven by mitochondrial antiviral-signaling protein (MAVS), as MAVS levels were also increased in NB-UVA treated CoV-229E infected cells [12]. Furthermore, the activation and potential cell-to-cell signal transmission/amplification of MAVS is a direct effect of exposure to NB-UVA [18], supporting our hypothesis that MAVS mediates NB-UVA-induced reduction of CoV-229E viral load.

MAVS is a key protein in early stages of RNA virus infections, leading to activation of downstream antiviral pathways, mostly driven by type I interferons (IFN I) [26]. However, many viruses, including SARS-CoV-2 [20], antagonize MAVS activation and evade IFN I-dependent immune responses [26]. It is now understood that SARS-CoV-2, in fact, does not primarily induce immune response through IFNs, but rather leads to expression of chemokines and cytokines, such as IL-6, TNF, IL-8, MCP-1 and MIG, resulting in cytokine storm or hypercytokinemia [9, 27]. Similar to SARS-CoV-2, here we found that coronavirus CoV-229E appears not to induce an immune response through IFNs (IFN-α2 and IFN-γ), but rather activates the expression of chemokines and cytokines including IL-6, TNF, IL-8, MCP-1, MIG, and at a later stage, IP-10. These results demonstrate that our *in vitro* model can, in part, mimic SARS-CoV-2 effects and downstream pathways associated with cytokine storm, consistent with previous findings by Lau et al [16], Loo et al [17], and Poppe et al [15]. Considering this, we explored the potential of NB-UVA exposure to ameliorate the cytokine storm that occurs following HTEpC transfection with CoV-229E.

We found that repeated NB-UVA exposure had a significant effect on several key secreted cytokines and chemokines that were upregulated during CoV-229E-induced cytokine secretion. Specifically, NB-UVA treatment significantly reduced secreted levels of IL-6 and TNF-α by at least 50%. These are two major cytokines associated with the activation of the systemic immune system and inflammatory responses [28], and are strongly correlated with COVID-19 severity and patient survival [10, 29]. Immune-mediated clearance of viral infections involves several orchestrated and well-balanced steps, starting with recognition of the pathogen, followed by cell recruitment and resolution of the damage [28]. During the resolution step, TNF-α induces vasodilation and wall

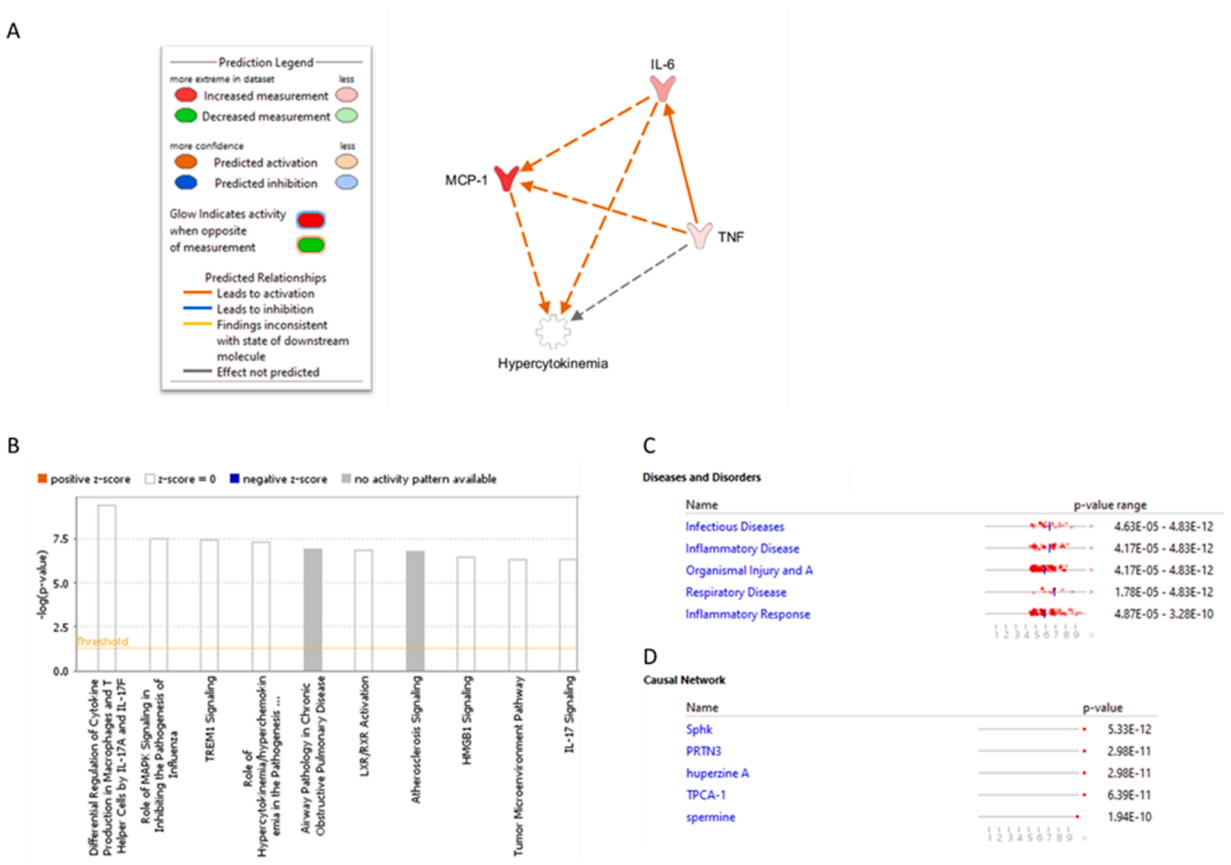

Fig. 8. A. Prediction network showing the interactions between cytokines/chemokines and activation of the hypercytokinemia (cytokine storm) pathway in CoV-229E transfected HTEpC that were treated 4 times with NB-UVA (120h). **B.** Top 10 canonical pathways predicted to be associated with the effects of NB-UVA on CoV-229E transfected cells, based on cytokine/chemokine patterns obtained *in vitro*. **C.** Top 5 diseases and disorders predicted to be associated with the effects of NB-UVA on CoV-229E transfected cells, based on cytokine/chemokine patterns obtained *in vitro*. **D.** Top 5 upstream regulators associated with the cytokine/chemokine patterns obtained in CoV-229E transfected HTEpC that were treated 4 times with NB-UVA.

permeability, allowing immune cells to reach the site of damage, while IL-6 induces complement and opsonization. However, the inflammatory response must be regulated to return to homeostasis, and levels of these biomarkers should be balanced to prevent uncontrolled systemic inflammation. A major surge in IL-6 and TNF-α production disrupts the delicate balance of a suitable inflammatory response, tipping it from being beneficial to destructive, through overactivation of immune cells and hyperregulation of proinflammatory markers [28], as is often seen in COVID-19 patients [27]. In our study it was evident that the levels of pro-inflammatory cytokines, including IL-6 and TNF-α, were significantly increased in CoV-229E transfected cells, but decreased in CoV-229E transfected cells after repeated therapy with NB-UVA light. Although more detailed mechanistic studies are needed, we hypothesize that this effect may be associated with a reduction in viral load caused by NB-UVA treatment, as previously published [12], promoting downregulation of pro-inflammatory cytokines, and leading ultimately to a balanced inflammatory response and proper resolution of infection.

Moreover, in addition to IL-6 and TNF-α, treating CoV-229E transfected cells with NB-UVA also had important downregulatory effects on secreted chemokines including MCP-1, IL-8, MIG and IP-10, the last three being reduced to levels similar to those in naïve control cells. Chemokines are relatively small bioactive molecules which mediate the activation and recruitment of immune cells to the site of infection, and further amplify the inflammatory response. Reduction in the levels of these biomarkers are associated, in most cases, with clearance of the infection and a subsequent return to homeostasis, including in COVID-19 patients [29]. These findings are supported by our results which suggest that repeated NB-UVA treatment results in an overall improvement in the inflammatory state in CoV-229E transfected endotracheal cells, resulting in increased cell survival [12].

While future studies on the effects of NB-UVA therapy on the cytokine storm driven specifically by SARS-CoV-2 are still needed, we have previously demonstrated a significant reduction in C-reactive protein (CRP) in critically ill COVID-19 patients following repeated treatments with NB-UVA[13]. CRP, an acute-phase reactant protein, is primarily induced by IL-6 [30] and has a regulatory effect on pro-inflammatory cytokines/chemokines, including TNF-α, IL-8 and MCP-1 [31].

This study has some limitations. The events identified *in vitro* may not represent the complexity of events *in vivo*. In addition, SARS-CoV-2 may not induce the same degree of immune response in the primary human tracheal cells used in this study as coronavirus CoV-229E, as immune pathway activation in response to coronavirus infection differs between various primary cells and cell lines [19]. We assessed the effects of UVA exposure at 24-hour intervals over 72-96 hours; however, the optimal timing and interval of UVA exposure to reduce inflammatory cytokines need to be determined in future studies.

In conclusion, this study shows that repeated exposure to NB-UVA may mitigate excessive immune system signaling by cells infected with coronavirus. It appears that NB-UVA exposure decreases the level of several pro-inflammatory secreted cytokines/chemokines in an *in vitro* model that mimics, at least in part, cytokine storm caused by coronavirus. These findings may explain some of the benefits of NB-UVA previously seen *in vitro* and in studies of critically ill human patients with COVID-19.

Declarations of Interest

Cedars-Sinai has a patent on internal UV therapy, inventors: AR, MP, GM, RM and GL. The rest of the authors declare no other financial conflicts of interest. Cedars-Sinai has a licensing agreement with Aytu BioSciences.

Funding

This study was supported in part by funds provided by the Monica Lester Charitable Trust and the Elias, Genevieve, and Georgianna Atol Charitable Trust in support of the MAST Program's COVID-19 research. The sponsors had no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Data statement

All data generated or analyzed during this study are included in the manuscript.

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