TLR7 and TLR9 Stimulation Can Protect from a SARS-CoV-2 Infection.

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), is a recently emerged respiratory coronavirus that has infected >100 million people worldwide with >2.1 million deaths in less than 1 year. Few COVID-19 specific therapeutics are available, and the basis for severe infections is not fully understood, however emerging data suggests that early anti-viral innate immune responses, particularly IFNs, play a critical role in the response. However, excessive inflammatory responses, particularly IL-6, at the time of diagnosis have been associated with more severe disease, leading to uncertainty regarding the use of immunomodulatory therapeutics for COVID-19. Previous studies have shown CpG ODN and other Toll-like receptor agonists can improve the response to multiple infectious agents by activating the innate immune system, mediated by the production of type I IFNs, and an increase in monocytes, Dendritic and natural killer cell activation. Here, we examined whether treatment with CpG ODN or other TLR agonists would improve the ability of immune cells to respond to a SARS-CoV-2 challenge. A screen of different TLR agonist shows that TLR7 and 9 agonists elicits a response by PBMC that protect susceptible ACE2-expressing cells from infection with SARS-CoV-2. The protective effect of these agonists is dose dependent and directly correlates with the expression of IFN-inducible genes and inversely correlates with IL-6. These studies suggest that TLR7 and 9 agonists may be effective as therapy for COVID-19 to prevent infection without eliciting excessive pro-inflammatory responses. While the proinflammatory effect of these therapeutics should be carefully monitored in clinical trials, our studies do not suggest that the risk of inflammation should preclude clinical studies.

Materials and Methods

Cell culture

Deidentified buffy coats were obtained from the NIH blood bank, (Bethesda, MD, USA). PBMC were isolated by density-gradient centrifugation over Ficoll-Hypaque. PBMC were cultured at 37°C in complete RPMI medium (10% FBS, NEAA, Na pyruvate, and HEPES) in 24-well plates at a density of $5x10^6$ cells/mL. Cells were stimulated with poly I:C (pI:C, 1μg/mL), Flagellin (Flag, 5μg/mL), Imiquimod (Imiq, 1μg/mL), CpGs D35 and K3 (1μM). Following 24 hours (gene expression) or 72 hours (protein) of TLR stimulation PBMCs or supernatants were collected and stored at -80°C.

HEK-Blue cells expressing human TLR9 and ACE2 were stimulated with K3 (0.3, 1, and 3μM) during infection with VSV-SARS-CoV-2 spike GFP expressing virus at a MOI of 0.1. 24 hours after infection level of viral infection was quantified by GFP expression in the each well. The degree of stimulation by K3 was assessed by measuring NFKB activation using the quanti-blue reagent.

CPE assay

VeroE6 cells were plated in a 96 multiwell plate at 1.8×10^4cells/well. 100 TCID50 of SARS-2 (isolate USA-WA1/2020) in 100ul MEM (2% FBS) was added with indicated amount of conditioned medium to the veroE6 cells. Virus was removed after 1 hour of incubation. Fresh media was added with conditioned media as indicated below and cells were incubated for an additional 72 hours. The CPE effect was measured using the CellTiter-Glo luciferase assay (Promega) was added according to the manufacture's recommendations.

Statistical significance in indicated on the graphs with * p<0.05, ** p <0.01 **** p <0.0001

Results and Discussion

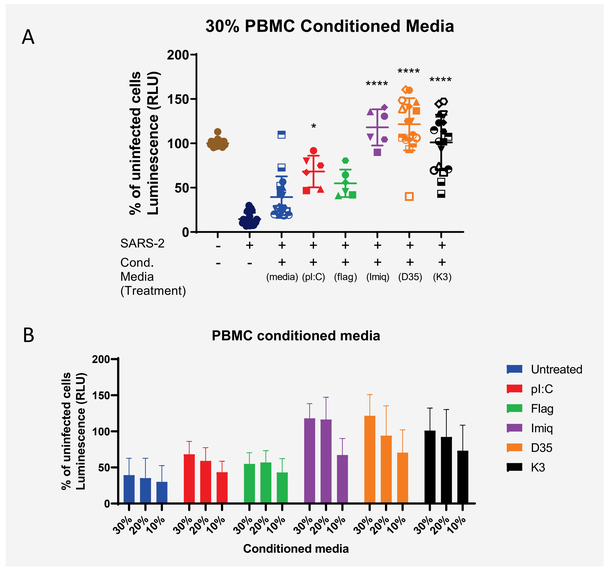

Figure 1. TLR stimulation by nucleic acids induced a protective environment in PBMCs conditioned supernatants.
Conditioned media from PBMCs stimulated with TLR ligands was added to VeroE6 cells infected with SARS-2. A) Imiq and CpG ODN (D35 and K3), and to a lesser extent pI:C treatment resulted in the protection of Vero cells from a SARS-2 infection. B) The protective effect of conditioned media was dose dependent. n = 6-16.

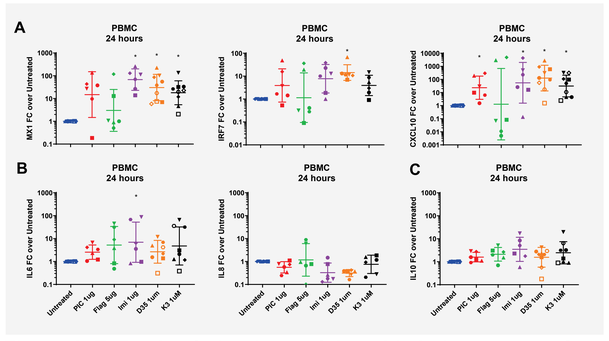

Figure 2. TLR stimulation by nucleic acids induces a type I IFN response.
Stimulation of PBMCs with Imiq and CpG ODN induced type I IFNs as indicated by the induction of MX1, IRF7, and CXCL10. There was a slight increase of proinflammatory cytokines following Flag and Imiq stimulation. n = 6-16.

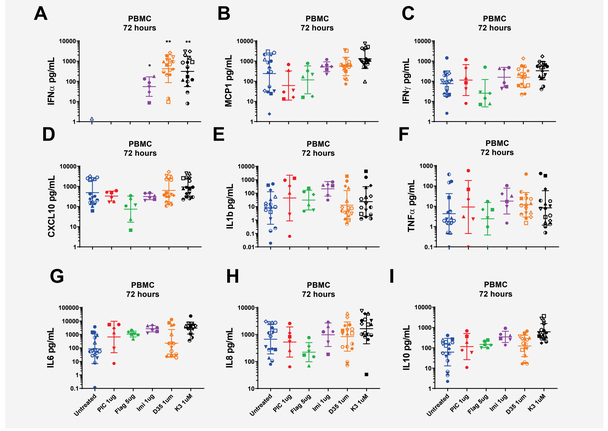

Figure 3. Cytokine induction in PBMCs following TLR stimulation.
Cytokine levels were quantified in conditioned media from PBMCs stimulated with TLR ligands for 72 hours. Imiq and CpG ODN treatment resulted in a significant secretion of IFNα (A). We did not observe a robust induction in other type I IFN responsive cytokines (B-D) possibly due to kinetics of the response. No inflammatory cytokines were significantly induced, but there was a trend towards more IL1β and IL6 in pI:C, Flag, and Imiq. n = 6-16.

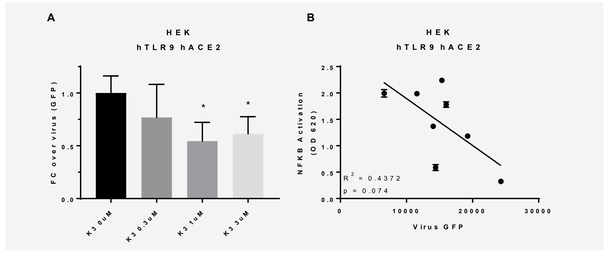

Figure 4. CpG stimulation protects HEK cells from VSV-SARS-CoV-2 spike infection.
HEK-Blue cells expressing hTLR9 and hACE2 were infected with a GFP expressing VSV-SARS-CoV-2 spike in the presence of increasing amounts of K3 ODN. A) Viral replication was measured by GFP fluoresces in infected cells 24 hours post infection. K3 stimulation protected cells in a dose dependent manner. B) The degree of NFkB activation was assessed in HEK-Blue using the SEAP reporter construct. A strong negative correlation of viral infection (GFP) and CpG stimulation (NFkB activation) was observed. n = 3.

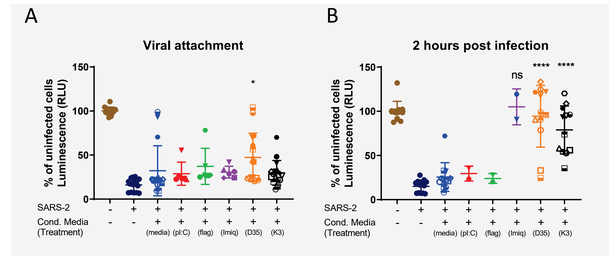

Figure 5. CpG ODN conditioned media can protect cell from viral infection at multiple points during infection.
Conditioned media was added to Vero during the viral attachment step or after infection. A) Only D35 treatment was able to significant protection cells during the attachment step, but the effect was milder than what was observed in other treatments. B) When conditioned media was added 2 hours after SARS-2 infection Imiq, D35 and K3 treatment protected Vero cells. N=2-12. ** p <0.01

Conclusion

In our current study we observed conditioned supernatants from PBMC stimulated by TLR7 or 9 ligands robustly protected Vero cells from infection by SARS-2. This effect appears to mediated through the induction of a type I IFN response as indicated by increased levels of IFNα and stimulation of interferon responsive genes. Importantly, these treatments also did not result in an increase in proinflammatory mediators such as IL1β, IL6 or TNFα which could potential make COVID19 more severe.

These results demonstrate the potential use of TLR7 and 9 stimulation as a pathogen agnostic therapy for our current pandemic and potentially other novel emerging pathogens.

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