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The Innate and Adaptive T cell Response to SARS-CoV-2

Sinead Cummins, Stefan Elekes, Kiran Singh, Derek Doherty
Trinity Translational Medicine Institute, St. James' Hospital, Dublin, Ireland

Background

- Understanding innate and adaptive immunity to SARS-CoV-2 is important for understanding the pathogenesis of COVID-19, vaccine development and pandemic response measures[1] .
- Critical (ICU) and fatal COVID-19 outcomes are associated with elevated levels of inflammatory mediators[2] and impairments in T cells.
- γδ T cells are thought to be involved in immunity against SARS-CoV-2 or pathogenesis of severe COVID-19:
  1. Severe COVID-19 patients have pronounced reductions in circulating γδ T cells.
  2. Evidence suggests activation of γδ T cells and infiltration of Vδ2 T cells in the lungs of COVID-19 patients[3].
- There are four major structural SARS-CoV-2 proteins: spike, nucleocapsid, membrane, and envelope (Figure 1).
- We investigated if the most immunogenic SARS-CoV-2 proteins, Spike and Nucleocapsid, can activate the 2 most abundant subsets of γδ T cells – Vδ1 and Vδ2 T cells, either directly or presented by dendritic cells (DCs).

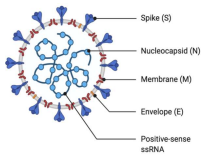


Figure 1: SARS-CoV-2 Structural Proteins

Aims

- Determine if DCs are activated by stimulation with spike and nucleocapsid peptides.
- Determine if γδ T cells respond to spike and nucleocapsid proteins presented by DCs.
- Determine the magnitude and nature of cytokine release in response to SARS-CoV-2 spike and nucleocapsid proteins by flow cytometry.

Methods

- γδ T cells lines and DCs were generated from peripheral blood mononuclear cells (PBMCs) isolated from blood packs from the Irish Blood Transfusion Service.
- The DCs were stimulated with SARS-CoV-2 spike protein and nucleocapsid protein from two sources (Raybiotech (RB) and Prof. Seamus Martin (SM)) and control stimuli (LPS, Curdlan, Poly I:C) and the expression of markers of antigen presentation and production of cytokines was analysed by flow cytometry.
- γδ T cells were co-cultured with SARS-CoV-2 stimulated DCs and analysed by flow cytometry for cytokine production.

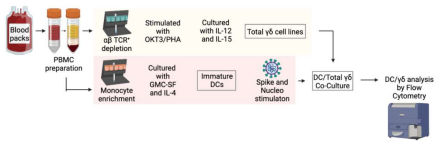
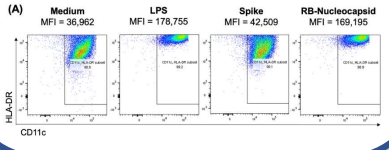


Figure 2: Method Summary Diagram

Results

- SARS-CoV-2 nucleocapsid, but not spike, protein induced weak expression of HLA-DR, CD40 and CD83, but not CD86 by DCs (Figure 3).
- SARS-CoV-2 nucleocapsid protein induced the production of IL-12 and TNF-α, but not IL-10 or IL-23, by DCs, however these increases were not significant (Figure 4).
- DCs pulsed with SARS-CoV-2 nucleocapsid, but not spike, protein significantly induced the production of IFN-γ, but not IL-17 or TNF-α by Vδ1 and Vδ2 T cells (Figure 5).
- The stimulatory effects of SARS-CoV-2 nucleocapsid protein for DCs and Vδ1 and Vδ2 T cells was not abrogated by digestion with V8 protease (Figure 6).
- The stimulatory effects of SARS-CoV-2 RB-nucleocapsid protein were not observed with a second source of nucleocapsid protein (SM-nucleocapsid) (Figure 7).
- Peptide fragments of SARS-CoV-2 spike and nucleocapsid that stimulate conventional T cells did not activate cytokine production by DCs or Vδ1 or Vδ2 T cells (Figure 8).



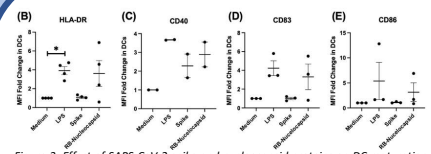


Figure 3: Effect of SARS-CoV-2 spike and nucleocapsid proteins on DC maturation. (A) Representative flow cytometry plots show HLA-DR expression by CD11c⁺ cells. (B-E) Scatter plots show the fold change in mean fluorescence intensity (MFI) of HLA-DR, CD40, CD83 & CD86 expression by DCs after each treatment over the MFI of these markers on unstimulated DCs (medium). MFI fold change was statistically compared using the Mann-Whitney U test.

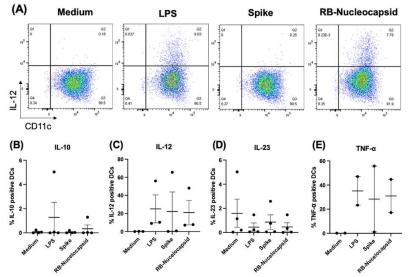


Figure 4: Effect of SARS-CoV-2 spike and nucleocapsid proteins on cytokine production by DCs. (A) Representative flow cytometry plots show IL-12 expression by CD11c⁺ cells (DCs). (B-E) Scatterplots show percentage positive IL10, IL-12, IL-23 & TNF-α DCs after treatment. Cytokine positivity is statistically compared using the Mann-Whitney U test.

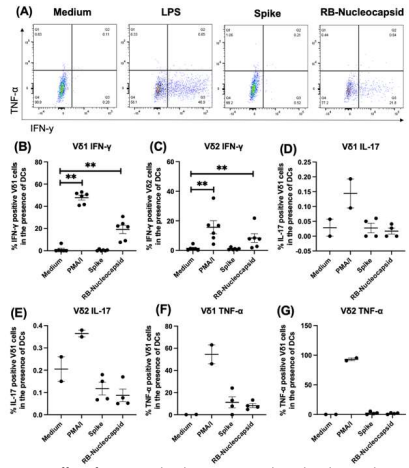


Figure 5: Effect of DCs treated with SARS-CoV-2 spike and nucleocapsid proteins on cytokine production by γδ T cells. (A) Representative flow cytometry plots show IFN-γ and TNF-α expression after gating on Vδ1 T cells. (B-G) Scatterplots show percentage positive IFN-γ, IL-17 or TNF-α cells for Vδ1 or Vδ2 T cells in the presence of DCs. Cytokine positivity of γδ T cells was statistically compared using the Mann-Whitney U test.

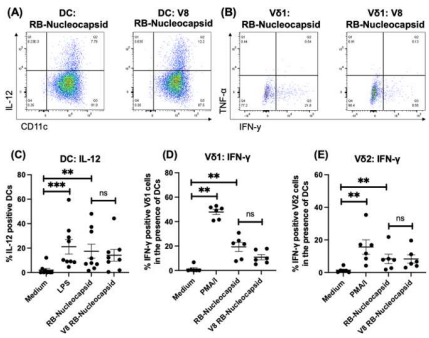
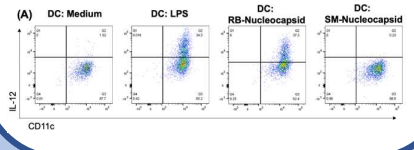


Figure 6: Effect of V8 protease treatment on stimulatory activity by RB-Nucleocapsid protein. (A) Flow cytometry plots showing IL-12 expression by CD11c⁺ cells after treatment with undigested and V8 protease-digested RB-nucleocapsid protein. (B) IFN-γ and TNF-α expression after gating on Vδ1 T cells after treatment with and without V8 protease. (C) Scatterplot shows percentage positive IL-12 DCs combined across all experiments. (D,E) Scatter plots show percentage IFN-γ positive Vδ1 and Vδ2 T cells. Cytokine positivity of cells was statistically compared using the Mann-Whitney U test.



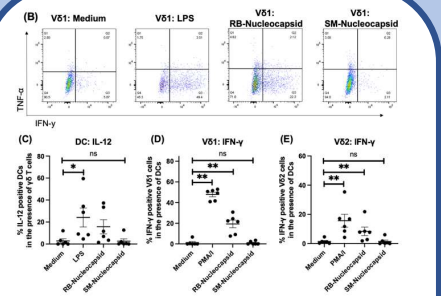


Figure 7: Effect of SARS-CoV-2 SM-Nucleocapsid protein on cytokine production by DCs and γδ T cells. (A) Representative flow cytometry plots show IL-12 expression by CD11c⁺ cells. (B) Flow cytometry dot plots show IFN-γ+ and TNF-α+ cells for Vδ1 T cells. (C) Scatterplot shows IL-12 positive DCs co-cultured with γδ T cells. (D-E) Scatterplots show percentage IFN-γ+ Vδ1 or Vδ2 T cells in the presence of DCs. Cytokine positivity of cells was statistically compared using the Mann-Whitney U test.

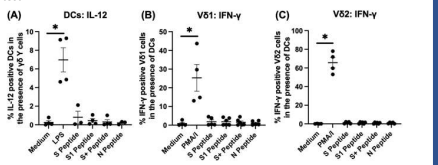


Figure 8: Effect of SARS-CoV-2 peptides on DCs and γδ T cell cytokine production. (A) Scatterplot showing percentage IL-12 positive DCs stimulated with pools of peptides derived from spike (S, S1 and S+) and nucleocapsid (N) proteins in the presence of γδ T cells. (B,C) Scatter plots show percentage IFN-γ positive Vδ1 or Vδ2 T cells in the presence of the peptide-pulsed DCs. Cytokine positivity was statistically compared using the Mann-Whitney U test.

Discussion

- Several studies have suggested that γδ T cells are involved in immunity against SARS-CoV-2. γδ T cells play crucial roles in frontline immunity against viruses through their rapid activation, cytotoxic response and cytokine release. They accumulate in the mucosal epithelia[4] and are depleted in severe COVID-19 patients[5].
- A previous study from our group showed that SARS-CoV-2 spike and nucleocapsid proteins do not directly activate γδ T cells.
- In the present study, SARS-CoV-2 nucleocapsid, but not spike protein induced DC maturation and cytokine production and these nucleocapsid-treated DCs promoted cytokine production by Vδ1 and Vδ2 T cells.
- However, protease digestion of the nucleocapsid protein did not abrogate its stimulatory activity, suggesting that the DC and γδ T cell activation by SARS-CoV-2 nucleocapsid is due to a non-protein contaminant, such as LPS.
- While SARS-CoV-2 nucleocapsid protein purchased from RayBiotech (RB-nucleocapsid) activated DCs and γδ T cells, treatment with SARS-CoV-2 nucleocapsid from a second source (SM-nucleocapsid) did not, further suggesting that the RB-nucleocapsid is contaminated with LPS.
- Sets of immunogenic SARS-CoV-2 spike and nucleocapsid peptides did not activate DCs Vδ1 or Vδ2 cells.

Conclusion

- SARS-CoV-2 spike and nucleocapsid proteins do not appear to activate DCs.
- SARS-CoV-2 spike and nucleocapsid proteins do not appear to activate γδ T cells in the presence of DCs.

References

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Acknowledgements

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