Multiparametric Investigation Of Spike-Protein Specific T-cell Cytokine Expression Profile In Children With Symptomatic COVID-19 Or Multisystem Inflammatory Syndrome

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BACKGROUND

Data regarding cell-mediated immunological differences in children across COVID-19 clinical spectrum are limited. We prospectively investigated Spike-protein specific cellular immunity in children with symptomatic COVID-19 or MIS-C, by single cell cytokine expression profiling.

METHODS

Peripheral blood mononuclear cells (PBMCs) were prospectively isolated from children with:

- MIS-C
- symptomatic COVID-19 (1 month after hospitalization)
- healthy controls

Cell suspensions were divided into two quantitatively equal samples (a negative control-unstimulated and a positive-stimulated).

Cells stimulation was performed using SARS-CoV-2 Spike antigenic peptides mix (Peptivator SARS-CoV-2 Prot_S). Cells of each sample were stained with fluorochrome monoclonal antibodies against 8 surface markers (CD3, CD4, CD8, CD14, CD19, CD137, CD197, CD45RA) and 6 intracellular markers (IL-4, IL-2, IL-17, IFN-γ, TNF-α, Granzyme B). Viability was assessed by 7AAD. Stained cell preparations were analyzed using 13 color Flow Cytometry (DX Flex, Beckman Coulter). Flow cytometric analysis was performed using Kaluza 2.1 Software.

RESULTS

Sixteen children (4 MIS-C, 8 post-COVID-19 and 4 healthy controls) were included in the study. The mean age (±SD) of the participants was 11.22 years (±3.48). **Children with MIS-C had significantly higher** mean (±SD) values of **CD8+IFN-γ/million CD3+** [226.68 (±134.92) vs 45.43 (±57.28); *P*: 0.033] and median (IQR) of **CD8+IFN-γ/ml** [156.38 (184.13) vs 26.34 (82.36); *P*: 0.033] compared to symptomatic COVID-19 children. Compared to healthy controls, MIS-C patients had significantly higher median (IQR) values of **CD4+IL-2/million CD3+** [923.12 (3777.95) vs 97.59 (71.71); *P*: 0.042] and **CD4+IL-2/ml** median (IQR) [626.33 (2744.98) vs 169.3 (173.91); *P*: 0.042]. No other significant differences were observed regarding other immunological markers in 3 study groups.

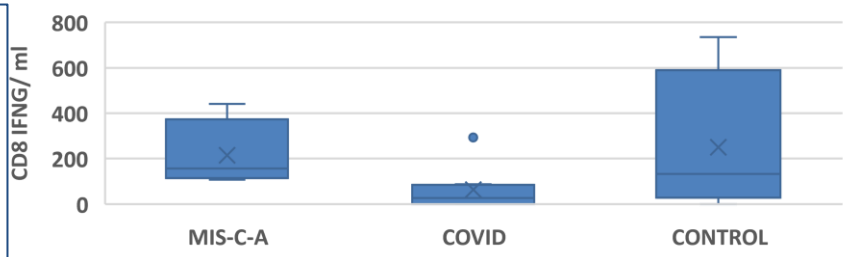

Fig 1: Boxplots of median (IQR) differences in CD8 IFN-g between the three study groups

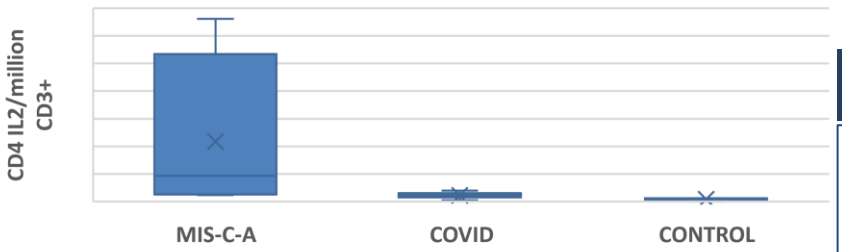

Fig 2: Boxplots of median (IQR) differences in CD4 IL-2 between the three study groups

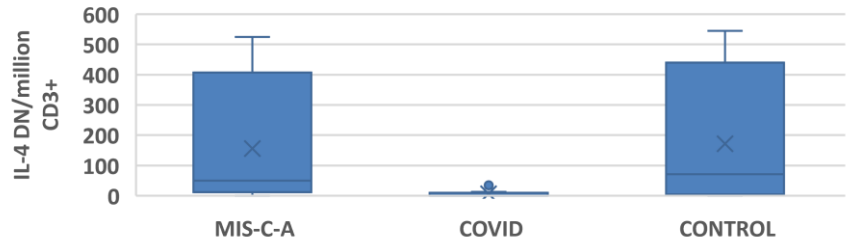

Fig 3: Boxplots of median (IQR) differences in IL-4 DN between the three study groups

CONCLUSIONS

IFN-γ production by CD8+ T-cells is highly expressed in children with MIS-C compared to hospitalized children one month after acute COVID-19 and could be a possible immunological biomarker. Further studies are needed in order to elucidate the pathophysiological basis of these findings.