Therapeutic and Protective Immunotherapy Potential of SARS-CoV-2-Specific γδ T Cells

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BACKGROUND

- COVID-19 is the most devastating pandemic in this century.
- Inconsistent and short-lived antibody responses observed in COVID-19 recovered individuals are concerning.
- It has been shown that gamma-delta (γδ) T cells played a significant role in past SARS epidemics. We have hypothesized and recently demonstrated that γδ T cells have a significant role against SARS-CoV-2.
- In this study, we evaluated their potential as therapeutic and protective cell-based immunotherapy.

METHODS

- Ten individuals with confirmed COVID-19 infection within the past 90 days of recruitment and seven seronegative individuals were recruited to the study. (Fig 1)
- γδ T cells were expanded *ex vivo* from pre- and post-infection PBMCs in the presence or absence of 100nM recombinant SARS-CoV-2 spike (S) protein.
- To assess their non-cytolytic antiviral activities, expanded cells were exposed to SARS-CoV-2-infected Vero cell culture supernatants. On day 4 after the exposure, γδ T cell culture supernatants were added to Vero cell cultures 24h before infecting them with SARS-CoV-2 (MOI 0.01). Cytopathic effects (CPE) of the virus were measured by daily cell count and imaging.
- Expanded γδ T cells were co-cultured 1:1 with infected (MOI 1) and uninfected ACE2-HEK cells to test their cytolytic antiviral activity. After 24h, target cell viability was measured by cell counter and flow cytometry. Co-culture supernatants were analyzed by a flow-based multiplex cytokine assay.
- Expanded γδ T cells were also assessed for markers for activation by CD25 and CD69, for cell exhaustion by CD57, and for antigen presenting cell (APC) functions by CD80, CD86, HLA-DR, CD11a, CXCR5, and CCR7 with flow cytometry.
- CD45RA-CD27- effector memory (EM) and CD45RA-CD27+ central memory (CM) subsets of expanded gd T cells were enriched by immunomagnetic separation and co-cultured with B cells harvested from seronegative individuals for assessment of their vaccine effects in vitro. Antibodies in co-culture supernatants were measured and evaluated for neutralization activity.

FIG. 1: PARTICIPANT DEMOGRAPHICS

COVID-19 Convalescent			Seronegative		
Participant #	**Gender**	**Age**	**Participant #**	**Gender**	**Age**
1	Male	44	11	Male	25
2	Male	36	12	Male	33
3	Male	26	13	Female	24
4	Female	34	14	Female	36
5	Male	48	15	Male	34
6	Male	27	16	Male	50
7	Male	51	17	Male	37
8	Female	24			
9	Female	35			
10	Female	29			

- Demographics of convalescent and seronegative trial participants.
- Pre- and post-infection samples from participants #1, #2, and #3 were evaluated

RESULTS

- Expansion rate of γδ T cells, and their EM and CM memory subsets were substantially higher in post-infection PBMCs compared to pre-infection and seronegative controls (Fig. 2).
- Vγ9Vδ2 T cell populations were selectively expanded in ex vivo cultures supplemented with S protein compared to other groups (Fig. 3).
- Memory subsets of ex vivo expanded SARS-CoV-2-specific γδ T cells displayed potent APC functions (Fig. 3).
- Virus-stimulated γδ T cell culture supernatants exhibited increased IFN-g, IL-10 levels and prevented CPE of virus at in vitro viral challenge (Fig.4).
- Isolated expanded γδ T cells effectively killed infected cells *in vitro* (Fig.5).
- In vitro vaccinated B cells demonstrated a robust production of SARS-CoV-2 neutralizing antibodies, predominantly in IgM and IgA isotypes (data not shown).

FIG. 2: 3/10 CONVALESCENT PARTICIPANTS gdT CELL PROFILE

- Expansion rate of γδ T cells, and their EM and CM memory subsets were substantially higher in post-infection PBMCs compared to pre-infection and seronegative controls. in participants #1, #2, and #3.

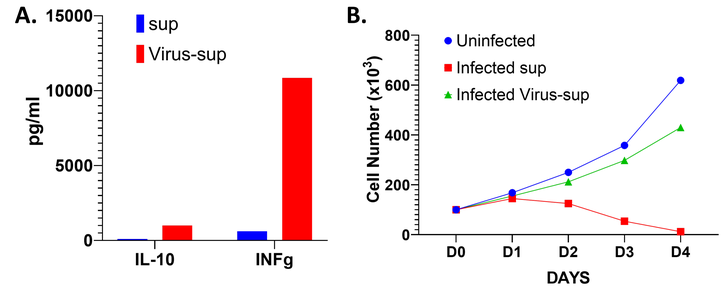
FIG. 3: PHENOTYPIC CHARACTERISTICS AND EXPANSION RATES OF γδ T CELLS

	Pre-Infection PBMC		Seronegative PBMC		Post-Infection PBMC	
Donors	3*		7**		10 (3* + 7**)	
Spike in the culture	No	Yes	No	Yes	No	Yes
Viability %	93 ± 5	92 ± 6	92 ± 7	91 ± 8	93 ± 6	95 ± 4
CD3⁺ %	91 ± 3	88 ± 4	90 ± 4	89 ± 5	91 ± 3	85 ± 4
Vγ9Vδ2 % in CD3⁺	87 ± 4	89 ± 3	90 ± 3	90 ± 3	91 ± 3	92 ± 5
Expansion fold	670 ± 112	691 ± 190	689 ± 201	701 ± 205	722 ± 189	1141 ± 128
CD69 % in Vδ2*	85	86	N/A	N/A	81	93
CD80 % in Vδ2*	91	92	N/A	N/A	80	88
CD86 % in Vδ2*	59	62	N/A	N/A	81	96
HLA-DR % in Vδ2*	91	92	N/A	N/A	96	99
CD11a % in Vδ2*	95	96	N/A	N/A	99	100

* Pre-infection and post-infection samples from donors #1, #2, and #3 were evaluated.
** Samples from seven seronegative individuals were evaluated

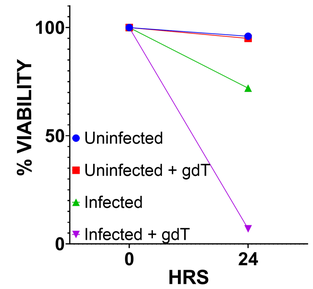
- Convalescent γδ T cells demonstrated a robust ex vivo expansion with S protein. They also displayed memory cell and APC functions.

FIG. 4: NON-CYTOLYTIC AND CYTOLYTIC ANTI-SARs-CoV-2 ACTIVITIES OF EX VIVO EXPANDED CONVALESCENT γδ T CELLS



- A: Virus-stimulated γδ T cell culture supernatants exhibited substantial increase in IL-10 and IFN-g levels.
- B: Virus-stimulated γδ T cell culture supernatants prevented CPE of virus in viral challenge (MOI 0.01) of Vero cells *in vitro*.

FIG. 5: SPECIFIC-KILLING OF ISOLATED AND EX VIVO EXPANDED SARs-CoV-2-SPECIFIC γδ T CELLS



- Isolated and ex vivo expanded SARs-CoV-2-specific γδ T cells successfully killed infected ACE2-HEK cells in vitro within 24 hours.

CONCLUSION

- SARS-CoV-2-specific γδ T cells harvested from convalescent individuals exhibited strong non-cytolytic and cytolytic antiviral activities, as well as substantial ex vivo expansion capabilities.
- Memory subsets of these cells displayed enhanced APC functions and elicited robust neutralizing antibody production in B cells harvested from seronegative individuals, suggesting not only therapeutic, but also potential protective immunotherapy implications of these cells.
- The findings support potential clinical use of these cells for treatment and prophylaxis of COVID-19.
- Our cell-based immunotherapy and vaccine candidates generated from SARS-CoV-2-specific convalescent γδ T cells are currently undergoing development and in vivo testing.

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