Journal of Applied Molecular Biology (JAMB)
Molecular biology Research & Studies institute , Assuit University, Egypt
ISSN 2974 – 4008
Volume 3 (1): January 2025

https://jamb.journals.ekb.eg

T Cell Comparing in COVID-19 Vaccinated Individuals with Non-Vaccinated Convalescent Patients

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ARTICLE INFO

Article History:
Received:25-09-2024
Accepted: 21-12-2024
Online: 27-01-2025

Keywords:
SARS-CoV-2; COVID-19; T cell, Immune response, COVID vaccine, Convalescent .

ABSTRACT

Worldwide, there are millions of afflicted individuals. by SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2, which is the cause of the COVID-19 global pandemic. reactions to vaccinations and the SARS-CoV-2 virus that trigger immunity. Numerous COVID-19 vaccines have been developed so quickly. Both cellular immunity, which consists of helper CD4+T cells and cytotoxic CD8+ T cells, and humoral immunity, which is mediated by antibodies and memory B cells, are responsible for producing protective immunity, which can be triggered by vaccination or infection. With minimal attention paid to cellular immunity, the majority of research on COVID-19 vaccines has been on neutralising antibody (NAb) responses. Mechanistic immunological correlates of vaccination protection as well as the duration, effectiveness, and type of immunity generated throughout SARS-CoV-2 infection are yet little known. Our research examined the response of SARS-CoV-2 T cells in 40 patients who recovered and in 40 more people who received the vaccine. Six to twelve months post-symptom onset (PSO), every patient exhibited measurable results of SARS-CoV-2-specific CD3+, CD4+, or CD8+ T cells; the immunisation group also showed similar outcomes. When we examined the T cell response in COVID-19-vaccinated individuals to non-vaccinated convalescent patients, we discovered that there were no statistically significant changes between the two groups in terms of overall T cell populations, namely CD3+ T cells, CD8+ Tc cells, and CD4+ Th cells. Our findings offer important proof that, in most patients, the response of T cells continues at least a year after an infection or immunisation.

1. INTRODUCTION

Since its inception in December 2019, the global pandemic of coronavirus (COVID-19) has been resulting from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1].

COVID-19 is transferred via respiratory particles; a virus generated in the respiratory secretions when a person with infection sneezes, talks, or coughs can spread to other people and infect them. if it is inhaled or touch surfaces with the infected mucous [2].

Viral vector vaccines (genetic material on adenovirus), whole virus (weakened, chemical or heat treatment) vaccines, protein-based vaccines (spike protein), and nucleic acid vaccines are the four main categories of COVID-19 vaccines, each utilizing a distinct platform.[3].

After the viral RNA enters the cell the translated proteins act as antigens, protein antigens are processed and presented on the antigen presented cell's surface for the activation and response of immune system. This response consists of helper T cells that facilitate the development of antibodies, B cells produce antibodies and mediated the humoral immune response, and killer T cells seek out and eliminate infected cells. [4]

T cells have already been identified by research as a potential key solution to the dilemma of exacerbation of COVID-19. A specific SARS-CoV-2 memory T cell phenotypes (effector memory for CD8 lymphocytes and central memory for CD4 lymphocytes) were seen in the patient's peripheral blood post symptom onset. [5]. limiting or preventing viral reinfection

Prevention of infection by COVID-19 and decrease the severity of the disease is still a worldwide priority. immunological protection can be reached either post infection or post vaccination, however the durability of the immune protection is a challenge. We evaluated the T cell response in COVID-19 vaccinee persons compared to non-vaccinated convalescent patients.

2. MATERIALS and METHODS

2.1. Methods:

Ethics statement: This research was accepted by the Research Ethics Committee of the Molecular Biology Research and Studies Institute (IORG0010947- MB-22-5-R), Assiut University, Assiut, Egypt.

During the period between January 2022 and December 2022, Our Cross-sectional study was done included 40 COVID-19 non-vaccinated Convalescent patients through past 6-12 months and 40 vaccinated non infected persons through the same duration, participants are from healthy hospital workers (doctors, nurses and employee) Assiut University Hospital

Data including personal name, age, sex, residence, and occupation was obtained. In addition to blood sample for complete blood count and flow cytometry analysis, which was done in South Egypt Oncology Institute, Assiut University**.**

Any patients with chronic liver disease, chronic kidney disease, known to have any type of malignancy or under immune suppression therapy were excluded from the study.

Written consent was obtained from each participant before enrollment in the study**.**

2.2. Reagents:

Fluorescein isothiocyanate (FITC) conjugated anti $CD3^+$(BD Bioscience), V450 conjugated anti $CD4^+$(BD Bioscience), Phycoerythrin (PE) conjugated anti $CD8^+$(BD Bioscience) ,Phosphate buffer saline (PBS) (10.7-gramNa_2HPO_4, 8.5-gram NaCl, 3.9-gram NaH_2PO_4, and 1-Liter distilled water, Lysing solution (8.3-gram NH_4CL, 0.0372-gram EDTA,1.0-gram $KHCO_3$ and 1 Liter Distilled water).

2.3. Isotype controls:

Cut-offs for positive subjects were determined by using isotype control staining as appropriate to exclude auto-fluorescence and non-viable cells that can cause non-specific fluorescence, which may be read as false positives.

Procedure of Preparation of the sample for examination by flow cytometry.

In a clean test tube one hundred microliters of blood. Mixed with Ten microliters of each of the monoclonal antibodies (CD3, CD4, CD8), Incubate for 15 minutes at 4°C in the dark. After incubation. Add 3ml of the RBCs lysing solution, then centrifugation was done at 2500 r.p.m for 1 min, the supernatant was discarded. The pellet washed in 3ml PBS; Centrifugation was done again at 2500 r. p.m. for l minute. the supernatant was discarded. Appropriate isotype controls were also prepared and processed in a similar manner without adding monoclonal antibodies. Acqusion on FACSCanto II (BectonDickinson, USA) Flow cytometric analysis was performed by FACSCanto II (BectonDickinson, USA) (BD), flow cytometry using FACSDiva 7.0 software (BD, USA). A minimum of 50,000 events were collected for each sample analysis.

2.4. Data management and analysis:

- Data were displayed as means, SD and median.
- Categorical variables were expressed as number (n) and percent (%).
- Comparison was done using a two-tailed unpaired t-test or two-tailed Mann-Whitney t-test.
- Pearson product-moment correlation Co-efficient, and Fisher exact test were used where appropriate P values of less than 0.05 were Confidence Statistically significant which means there is a differences between vaccinated and covid convalescent as in Figure 1.

3. RESULTS

The study includes:

A. 40 vaccinated non infected persons. Their age range was 19-51 years (median=24 years).

B. 40 COVID-19 non-vaccinated Convalescent patients. Their age range was 23-60 years (median=36.5 years)

Demographic and Comorbidity Data of Vaccinated and Convalescent Groups as shown in table (1).

Both total lymphocytic and Monocytic count are statistically significant lower in the convalescent group in comparison to the vaccinated group.

However, the granulocytic count is statistically higher in the convalescent group.

Other criteria in complete blood count like white blood count, red blood count and Platelets show no statistically significant differences between vaccinated and convalescent individuals as shown in table (2).

After the flowcytometric tests (CD3, CD4 and CD8) between vaccinated and convalescent groups as shown in table (3).

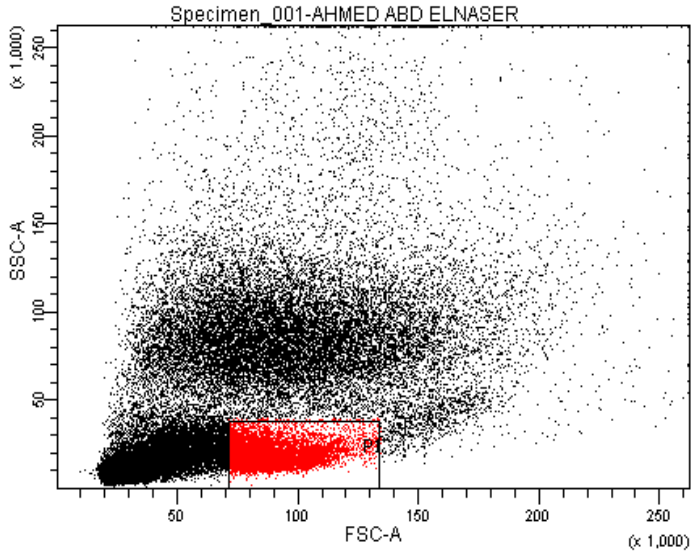

Figure 1. The lymphocytes population was selected by drawing (P1) on the forward scatter (FSC) /side scatter (SSC) dot plot

There was notable that, No statistically significant variations in the total T cell population. CD3+ T cell, CD8+T cell and CD4+ T cell between vaccinated and convalescent individuals. As in **Figure** (2)

There is no positive or negative significant correlation between CD3, CD4 and CD8 and all parameters of the CBC in both vaccinated and convalescent groups except weak positive significant correlation between Platelet count and CD3 level in vaccinated group only.

Table 1. Demographic and Comorbidity Data of Vaccinated and Convalescent Groups

Demographic data	Vaccinated N=40	COVID convalescent N=40
Age range	19-51 (median =24)	23-60 (median=36,5)
Female	25 (62.5%)	31 (77.5%)
Male	15 (37.5%)	9 (22.5%)
Smoking	1 (2.5%)	2 (5%)
Diabetes	0	2 (5%)
Hypertension	0	3 (7.5%)
Other Co-morbidities	0	2 (5%)

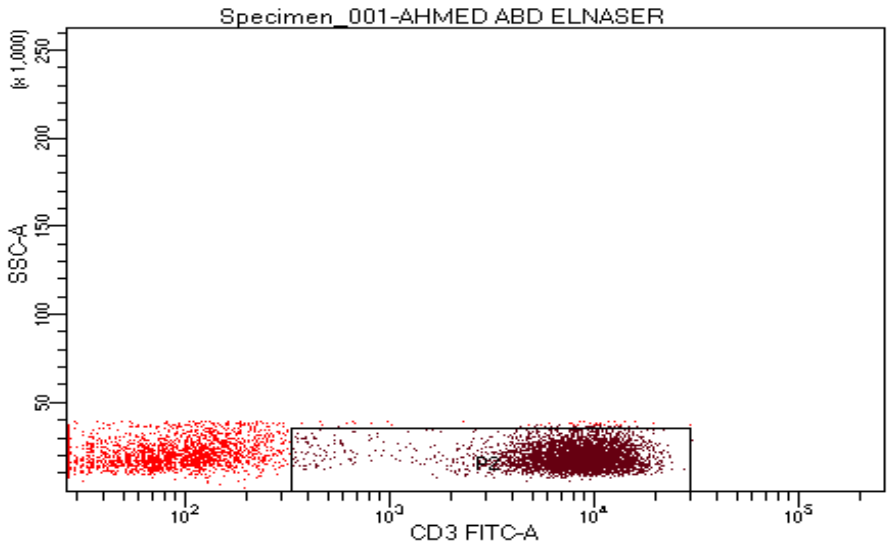

Figure 2. Then (P2) region was drawn to select CD3+ Tcell

Table 2. Comparison of Complete Blood Count (CBC) Parameters Between Vaccinated and Convalescent Groups

Item	Vaccinated N=40	COVID convalescent N=40	P value
White blood count (WBC)	6.3350 ±1.61397	7.7225 ±5.16107	0.109
Red blood cell (RBC)	14.4343 ±60.25823	4.6232 ±.40646	0.306
Hemoglobin (HGB)	13.0125 ±1.62736	12.2975 ±1.64652	0.054
Hematocrit (HCT)	39.8200 ±4.60224	38.1800 ±4.25159	0.102
Mean Corpuscular Volume (MCV)	82.5200 ±6.78253	82.3200 ±8.82174	0.91
Mean Corpuscular Hemoglobin (MCH)	26.7825 ±2.77645	26.6975 ±3.39166	0.903
Mean Corpuscular Hemoglobin Concentration (MCHC)	32.4450 ±1.12636	32.1375 ±1.15774	0.232
Platelets (PLT)	278.825±83.83864	299.225±79.02693	0.266
Lymphocytes (LY)	47.6725 ±10.74829	41.9450 ±12.96391	0.035
Monocytes (MO)	5.8550 ±2.27370	4.3650 ±2.24346	0.004
Granulocyte (GR)	45.9675 ±12.46452	53.6775 ±13.96765	0.011
Red blood cell distribution width (RDW)	13.9725 ±16.88896	11.6500 ±1.84363	0.39
Platelet Crit (PCT)	0.2018 ±.05719	0.2240 ±.10970	0.259
mean platelet volume (MPV)	7.4300 ±1.41298	6.9175 ±1.34105	0.1
Platelet distribution width (PDW)	17.3300 ±.77433	17.4850 ±1.15970	0.484

Table 3. CD3+T cell, CD4+T cell and CD8+T cell comparison between Vaccinated and Convalescent Groups

	Vaccinated N=40	COVID convalescent N=40	P value
Lymphocytes %	24.8925 ±7.14367	22.600± 7.18499	0.156
CD3+ T cells %	69.4000 ±9.26311	70.9825± 11.47566	0.499
CD3+ CD4+ T Helper cell %	52.8100 ±8.09482	53. 6875 ± 6.95152	0.061
CD3+ CD8+ T Cytotoxic cell %	31.9375± 8.79748	35.4275± 7.60920	0.149

Table 4. Correlation between CD3, CD4, CD8 and CBC parameters

		Vaccinated (N=40)			Covid convalescent (n=40)		
		CD3	CD4	CD8	CD3	CD4	CD8
WBCs	r P	-.163 .314	.314 .906	-.105 .518	.088 .589	-.011 .945	.136 .404
LY	r P	-.092 .570	.207 .200	.136 .404	.181 .263	.030 .852	-.271 .091
Monocyte	r P	-.192 .235	.223 .166	-.139 .391	-.227 .159	-.037 .820	-.162 .318
Granulocyte	r P	.015 .927	-.169 .298	-.221 .171	-.132 .418	-.023 .887	.279 .082
HGB	r P	-.221 .170	-.293 .066	.020 .901	-.033 .842	-.107 .511	-.226 .160
PLT	r P	.327 .039	-.124 .447	.142 .383	.050 .760	.107 .512	.026 .871

4. DISSCUSION

The SARS-CoV-2 pandemic has a major effect around the world. It is crucial to understand the processes behind SARS–CoV–2–targeted immunological reactions to facilitate the discovery of therapeutic and protective methods against COVID–19.

T cell immunity is critical during COVID-19, as demonstrated by a collection of studies on patients and convalescents that focuses on the frequency, diversity, and extent of T cell responses and how they relate to SARS-CoV-2 antibody titers and clinical features [6].

The adaptive immune system is the mediating factor for long-term immunity. When exposed to the same virus infection, memory T cell and B cell facilitate more effective and rapid defenses. [7] T cell memory is a key for vaccination development for long-term immune protection following COVID-19 infection or vaccination.

CD4+ T cells are essential for cellular immunity against SARS-CoV-2, according to several research examined the responses of SARS-CoV-2 T cell throughout or immediately following COVID-19 infection. The response of T cell strength and cytokine profile of CD4+ T cells vary more than those of CD8+ T cells. **[**8]

Following COVID-19, an extensive "inflammatory state" characterized by periodic activation of several immune system components has been reported. [9] This may cause continuous T cell activation by various immune system components, This is necessary for developing and persistence of memory CD4+ T cells. [10]

Increased cross-reactive CD4+ T cell and SARS-CoV-2-specific responses may be explained by the interaction of B and T cells, which may be crucial. [11]

The information provided by Dan et al.. [12], exhibiting a tendency to grow in Specific follicular helper CD4+T cells for SARS-CoV-2, a particular fraction of CD4+ T cells needed for B cell assistance [13], half a year following infection. As in **Figure 3**

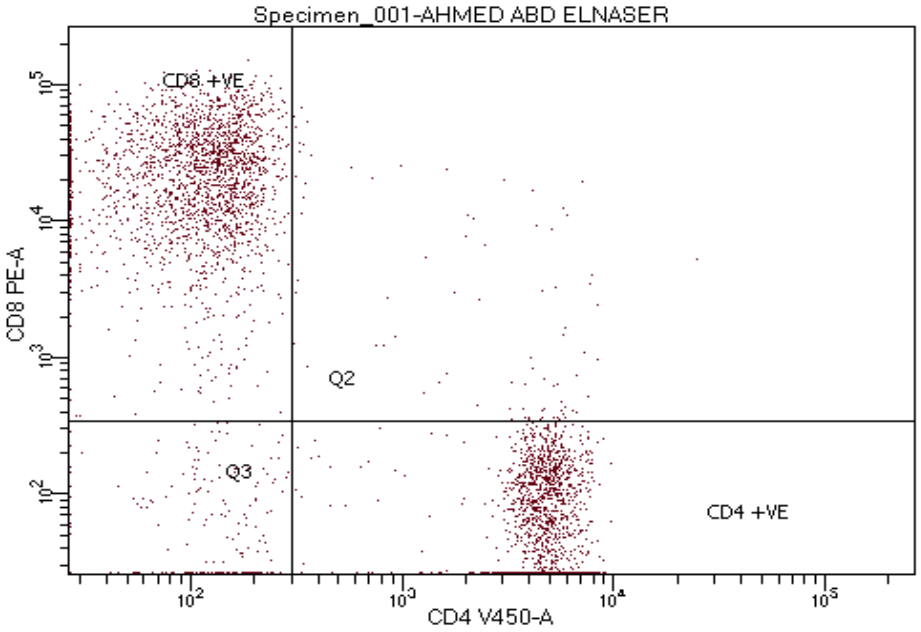

Fig (3). Percentages of CD8+ and CD4+ T cells were then detected

Accurate information about the dynamics of vaccine-induced immune responses against newly emerging SARS-CoV-2 variants is necessary for the development of efficient vaccines and prevention and control measures. Despite earlier research showing a decrease in neutralizing the levels of antibodies against immune-escape-mutant variants of SARS-CoV-2 [14].

According to epidemiological data, vaccine efficacy toward symptomatic [15] or serious illness and hospitalizations [16] demonstrating immune defense by cellular mechanisms.

In this research, we present a study about the immune level of SARS-CoV-2 CD8+ and CD4+ T cells following vaccination, as well as upon COVID-19 infection, and the ability of T cell responses to endure for at least six months following vaccination. Fortunately, all blood samples were freshly examined. We optimized the activation induced **markers** (AIM) protocol was applied with a stimulation duration limit of 48 hours (about 2 days).

In other study that made on CD4+ T cell responses directed against spike-protein there is no statistically notable variations in between immunized and recovering people. [17,18].

In other research that made on activated CD8+ T cells are few in the blood and tend to be scattered throughout the body after infection, it has been challenging to research CD8+ T cell responses [19], lowering the memory CD8+ cell count in circulations [20]. This could make understanding the relationship between the cell-mediated defense **against** COVID-19 and the CD8+ T cell findings from mononuclear cells from peripheral blood even more difficult.

According to our data, vaccination recipients' and convalescent patients' samples showed persistent CD4+ and CD8+ T cell. examining how long COVID-19 vaccine-induced immunity lasts against the current and potential future strains of concern is crucial.

According to some research, there was no discernible difference in responses of T cells between people who were asymptomatic and those who had symptoms. The T cell immune responses to COVID-19 instances either reduced or did not differ in severity. [21]. Tegeler [22] reported that severe COVID-19 symptoms are avoided by quick virus clearance facilitated by SARS-CoV-2-specific T cells.

Furthermore, T cell immunity is particularly important for the immune system's defense during COVID-19; research has indicated that T cell responses can occur during acute infection and lasting as long as eight months following recovery [6]. Evidence for possible preexisting immunity against human coronaviruses that cause the common cold, mediated by T cells which are cross-reactive, also supports this [18].

The T cell epitopes causing these distinct and cross-reactive T cell responses to SARS-CoV-2 have been identified in recent research, both in convalescent and unexposed individuals. This indicates that multiple epitope identification is necessary for the development of immunity [18]. Continued protection of T cells immunity specific to SARS-CoV-2 is essential for long-term protection following COVID-19, that has significant implications for development of vaccines given the information currently available on immunological responses against SARS-CoV-2..

This research has some limitations, such as a small number of samples, a limited follow-up, and a particular focus on COVID-19 mild courses. Since the lasting continuation of SARS-CoV-2 immunological responses in COVID-19 cohort recovering donors is vital to the establishment of immunity at the population-level. And that we measure the whole CD4+ and CD8+ T cells so the level of follicular helper or memory T cell separately are not measured [23].

CONCLUSION

A new coronavirus is the cause of the global epidemic of severe acute respiratory syndrome, or COVID-19. SARS-COV2 causes an adaptive immune activated response that is mediated by B and T cells. Excitation of T cells is mediated by fragments of viral proteins which in turn contribute to viral clearance but may also promote the development of cytokine storm and inflammation-induced severe lung damage.

In our results we studied CD3+ T cell, CD8+ T cell and CD4+ T cell after vaccination, and convalescent, we did not find statistically significant changes between the vaccinated and convalescent individuals.

Our findings showed that most samples from both vaccination recipients and patients in the convalescent period had long-lasting immunity targeting CD4+ and CD8+.

Examining how long COVID-19 vaccine-induced immunity lasts against the current and potential future strains of concern is crucial to reduce the risk of infection, general prevention and containment precautions are required.

In conclusion, our information offers vital details about T cell immunological responses to SARS-CoV-2, their ratio post–COVID-19 illness. When combined, these data have wide consequences regarding both identification and know more about long human defense mechanisms memory for virus as well as vaccine desig

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