Communication

Analysis of Cell Immunity for Children Infected with COVID-19 and Those Vaccinated against COVID-19 Using T-SPOT®.COVID

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Analysis of Cell Immunity for Children Infected with COVID-19 and Those Vaccinated against COVID-19 Using T-SPOT®.COVID

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Abstract: To elucidate the cellular immune response to coronavirus disease (COVID-19) among children, we assessed cellular immunity in 8 children post-vaccination for COVID-19 and 11 children after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, using the T-SPOT®.COVID assay for both the spike (S) and nucleocapsid (N) proteins. In the vaccinated group, the T-SPOT®.COVID assay for the S protein yielded positive results in all eight children. In contrast, in the post-infection group, the assay for the N protein was positive in 5 of 11 children, with 3 of these 5 children requiring hospital admission, including 2 who needed mechanical ventilation. Therefore, the T-SPOT®.COVID assay is valuable for assessing cellular immunity against COVID-19, and most children infected with COVID-19 may not develop such immunity unless the disease severity is significant.

Keywords: cell immunity; COVID-19; children; T-Spot®

1. Introduction

Immune responses such as humoral immunity and cell immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are induced by vaccinations against coronavirus disease (COVID-19) or by SARS-CoV-2 infection [1,2]. In humoral immunity, the sensitivity of antibody neutralization reportedly decreases for the SARS-CoV-2 variant [3]. Meanwhile, cellular immunity has been shown to be effective against new SARS-CoV-2 variants [4]. However, unlike humoral immunity, which involves antibodies, measuring cellular immunity is challenging owing to the large volume of blood samples required and immediate processing needed for lymphoid cell separations. This is particularly difficult in pediatric patients. Therefore, only few reports are available on cell immunity against SARS-CoV-2 for children infected with COVID-19 [5,6].

To evaluate cellular immunity against SARS-CoV-2, a commercial kit, namely the T-SPOT®.COVID (Oxford Immunotec, Oxfordshire, in United Kingdom) may be useful because it can be used in many medical settings [7]. Although an evaluation of cellular immunity against SARS-CoV-2 using this kit for adults has been reported [8], to the best of our knowledge, no studies have investigated such use in children. Therefore, in this study, we aimed to analyze the cellular immunity against SARS-CoV-2 in children using the T-SPOT®. kit to elucidate the relationship between cellular and humoral immunity following COVID-19 vaccination or diagnosis.

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2. Materials and Methods

2.1. Ethical Aspects

Informed consent was obtained from children or their parents. The study protocol was approved by the Ethics Committee of Kawasaki Medical School, Kurashiki, Japan, on August 1, 2023 (no. 5370-04).

2.2. Sample Collection

In our study, we analyzed blood samples isolated from children vaccinated for COVID-19 or with prior COVID-19 diagnosis. These samples were collected from children at the children's ward or pediatric clinic of Kawasaki Medical School Hospital. Furthermore, we recorded the age, sex, medical histories, frequencies of COVID-19 diagnosis or vaccination, and the time elapsed since COVID-19 diagnosis or vaccination.

2.3. SARS-CoV-2 Antigen-Specific T cell Responses

We assessed SARS-CoV-2 antigen-specific T cell responses after COVID-19 and vaccination for COVID-19 using the T-SPOT®.COVID kit [7]. These assays identify SARS-CoV-2 antigen-specific interferon-gamma-secreting T cells, primarily consisting of CD4+ T helper type 1 cells and CD8+ cytotoxic T cells, essential for an antiviral immune response. Furthermore, peripheral blood mononuclear cells are stimulated with overlapping peptide pools of SARS-CoV-2 spike subunit 1 (S1), nucleocapsid protein (N) in these assays. Peptides high homologous to endemic coronaviruses were omitted from peptide pool for S1 and N in T-SPOT®.COVID to minimize the chance of T-SPOT.COVID responses being elicited by cross-reactive T cells in the absence of SARS-CoV-2 infection [7]. Blood samples for the T-SPOT®.COVID test were drawn into heparin tubes and subsequently shipped to LSI Medience Corporation (Tokyo, Japan) in temperature-regulated boxes.

The T-SPOT®.COVID test were processed and analyzed according to the manufacturer's instructions.

2.4. Serum Immunoglobulin (Ig)G and ELISA for SARS-CoV-2

Anti-SARS-CoV-2 antibodies were measured using the Elecsys® Anti-SARS-CoV-2 RUO® assay (Roche Diagnostics, Basel, Switzerland), which is based on the modified double-antigen sandwich immunoassay with recombinant protein representing the nucleocapsid antigen that measures the total antibody against SARS-CoV-2 (panimmunoglobulin). The assay was performed using a fully automated Cobas e801 analyzer (Roche Diagnostics) as described in a previous study [9].

3. Results

Table 1 presents data on demographics, T-SPOT®.COVID, and IgG for SARS-CoV-2 in 8 children were vaccinated against COVID-19. Their ages ranged from 7 to 15 years, comprising six males and two females. The interval from the last vaccination to sample collection varied between 9 days and 4 months, with vaccination frequencies ranging from once to three times. Two participants had been diagnosed with COVID-19 prior to sample collection.

Table 1. Children after COVID-19 vaccinations (April–December 2022）.

Age (Year-old)	Sex	Date of collection (month/year)	Date of last vaccination	Frequency of vaccination	Onset of COVID-19	T-SPOT®.COVID targeting S proteins (spot) *1	T-SPOT®.COVID targeting N proteins (spot) *1	SARS-CoV-2 IgG to S proteins (U/mL) *2	SARS-CoV-2 IgG to N proteins (U/mL) *2	Note
7	-M	May/2022	3 weeks prior	1	-	0 (-)	0 (-)	19.6 (+)	<0.8 (-)	

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		August/2022	2 months prior	2	3 weeks prior	40 (+)	14 (+)	570 (+)	6.2 (+)	
11	M	June/2022	9 days prior	1	-	35 (+)	4 (-)	78.5 （＋）	16.0 （＋）	Close contact 1 month prior
		July/2022	1 month prior	3	-	32 (+)	7 (±)	71.3 (+)	17.6 (+)	
9	M	July/2022	2 weeks prior	2	-	≥50 (+)	4 (-)	1760 (+)	38.0 (+)	
13	F	June/2022	2 weeks prior	3	-	22 (+)	0 (-)	-	-	
		September/2022	3 months prior	3	-	11 (+)	1 (-)	1490 (+)	<0.8 (-)	
15	M	August/2022	4 months prior	3	-	7 (±)	0 (-)	8330 (+)	<0.1 (-)	
13	M	August/2022	2 months prior	3	-	23 (+)	-1 (-)	999 (+)	<0.8 (-)	
14	M	August/2022	4 months prior	3	2 months prior	12 (+)	3 (-)	11000 （+）	3.9 （＋）	
15	M	November/2022	2 months prior	3	-	41 (+)	1 (-)	542 (+)	<0.8 (-)	
Mean: 13 （7–15）	M: 6 F:2	May–November/2022	3 days prior -4 month prior	1-3	Positive: 9/	Positive: 9/11 Equivocal: 1/11 Negative: 1/11	Positive: 1/11 Equivocal: 1/11 Negative: 9/11	Positive: 10/10 Equivocal: 0/10 Negative: 0/10	Positive: 5/8 Equivocal: 0/8 Negative: 3/8	

*1: ≥8 spots, positive (+); 5–7 spots, not determined (±); ≤ 4 spots, negative (-); *2: ≥0.8 U/mL, positive (+); <0.8 U/mL, negative (-); SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; IgG, Immunoglobulin G; S, S proteins; N, N proteins; F, Female; M, Male.

T-SPOT ®.COVID targeting S proteins was positive in seven of eight children; the remaining one was not determined. In contrast, the T-SPOT ®.COVID targeting N proteins was positive in only one individual who had a history of COVID-19. Among those tested for antibodies to SARS-CoV-2, seven were positive for IgG to the S protein and negative for IgG to the N protein, except for the individual with a history of COVID-19.

Data from the 11 children with a history of COVID-19 are shown in Table 2. Their ages ranged from 1 to 14 years old, and there were four males and seven females. The duration from the onset of COVID-19 to sample collection ranged from 3 days to 4 months. Two individuals had been vaccinated prior to contracting COVID-19. Five were admitted to the hospital, two of whom required mechanical ventilation.

Table 2. Children with COVID-19 (April–December 2022）.

Age (Year-old)	Sex	Date of collection (month/year)	Onset of COVID-19	Date of last vaccination	T-SPOT®.COVID targeting S proteins (spot) *2	T-SPOT®.COVID targeting N proteins (spot) *2	SARS-CoV-2 IgG to S proteins (U/mL) *3	SARS-CoV-2 IgG to N proteins (U/mL) *3	Note
6	F	May/2022	5 days prior	-	2 (-)	4 (-)	<0.8 (-)	<0.8 (-)	Hospitalization
		June/2022	13 days prior	-	5 (±)	5(±)	-	-	

 doi:10.20944/preprints202403.1033.v1

4	M	July/2022	3 days prior	-	0 (-)	0 (-)	<0.8 (-)	<0.8 (-)	Hospitalization
		July/2022*1	3 weeks prior	-	2 (-)	3 (-)	4.3 (+)	1.4 (+)	
11	F	July/2022	4 months prior	-	2 (-)	0 (-)	1.2 (+)	<0.8 (-)	
9	F	July/2022	4 days prior	-	6(±)	7 (±)	-	-	Hospitalization
		August/2022	2 weeks prior	2 months prior	40 (+)	14(+)	570 (+)	6.2 (+)	
7	M	August/2022	2 months prior	5 months prior	12(+)	3 (-)	11000 (+)	3.9（＋）	
14	M	August/2022	2 weeks prior	-	19(+)	27(+)	5.32 （＋）	4.6（＋）	Mechanical ventilation
3	F	August/2022	12 days prior	-	20(+)	30(+)	<0.8 (-)	2.5 (+)	Mechanical ventilation
1	F	September/2022	1 month prior	-	3 (-)	3 (-)	-	-	
11	M	September/2022	3 weeks prior	-	3 (-)	3 (-)	-	-	
11	F	December/2022	1 month prior	-	2 (-)	7 (±)	-	-	
9	F	May/2022	5 days prior	-	2 (-)	4 (-)	<0.8 (-)	<0.8 (-)	
Mean: 9（1–14）	M: 4 F:7	May–November/2022	3 days prior to 4 month prior		Positive: 4/13 Equivocal: 1/13 Negative: 8/13	Positive: 2/13 Equivocal: 3/13 Negative: 8/11	Positive: 5/8 Equivocal: 0/8 Negative: 3/8	Positive: 5/8 Equivocal: 0/8 Negative: 3/8	

*1: Approximately 3 weeks interval between *the* first and second collections. *2: ≥8 spots, positive (+); 5–7 spots, not determined (±); ≤ 4 spots, negative (-); 3: ≥0.8 U/mL, positive (+); <0.8 U/mL, negative (-); SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; IgG, Immunoglobulin G; F, Female; M, Male; S, S proteins; N, N proteins.

The T-SPOT ®.COVID targeting S proteins yielded positive results in 4 of 11 children, indeterminate results in 2, and negative results in 5. Regarding the T-SPOT ®.COVID targeting N proteins, the results were positive in three cases (all of whom were also positive for S proteins), indeterminate in three, and negative in five. Among seven children, eight tests for SARS-CoV-2 IgG to both S and N proteins were conducted: five tests were positive for IgG to S proteins, and similarly, five were positive for IgG to N proteins. Three tests were negative for both.

4. Discussion

We analyzed cell immunity against COVID-19 among children using the T-SPOT®.COVID kit along with humoral immunity against COVID-19. To the best of our knowledge, this is the first study evaluating cell immunity against COVID-19 after COVID-19 vaccination and diagnosis among healthy children using the T-SPOT ®.COVID.

Nearly all children vaccinated against COVID-19 tested positive for T-SPOT®.COVID targeting S protein. Research indicates that cellular immunity against COVID-19 persists long after vaccination in adults [10]. Similarly, we demonstrated that children can acquire cellular immunity against COVID-19 through vaccination. However, many children who contracted COVID-19 tested negative for T-SPOT®.COVID targeting both the S and N proteins. We hypothesized that the limited positive

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cellular immunity observed in children post-infection in our study may be attributed to the mild severity of COVID-19 in children. In most cases, children with COVID-19 experience mild symptoms [11]. Furthermore, primed innate immunity among children is reportedly useful for fighting SARS-CoV-2 infection [12]. Hence, cell immunity might be unnecessary among many children with mild cases of COVID-19. Notably, two hospitalized children who needed mechanical ventilation, indicating severe COVID-19 cases, exhibited positive results for T-SPOT®.COVID targeting the S and N proteins. A previous report indicated that in children with COVID-19, the innate immunity response was higher than in adults, showing an inverse correlation with the severity of COVID-19 [13]. This knowledge lends support to our hypothesis. Therefore, we believe that while children can acquire cellular immunity against COVID-19 through vaccination, in many cases of infection, this immunity may not be adequately developed.

Further, we analyzed humoral immunity against COVID-19. Because all children after COVID-19 vaccinations were able to develop SARS-CoV-2 IgG to S proteins, COVID-19 vaccinations in this age group are thought to be useful. SARS-CoV-2 IgG to N proteins were positive in three of them, and the history of COVID-19 was not clarified in two of these three. Therefore, asymptomatic COVID-19 might also occur in children. Meanwhile, among children who had a COVID-19 history, some had positive SARS-CoV-2 IgG even when the T-SPOT®.COVID result was negative. This discrepancy is thought to be due to insufficient cellular immune responses in children, caused by either the immaturity of cellular immunity or the severity of COVID-19. Regarding the relationship between the severity of COVID-19 and immunity, cellular immunity levels against COVID-19 were higher in adults with severe COVID-19 than in those with milder cases. In contrast, in our study, children hospitalized due to COVID-19, indicating more severe cases, tended to have a higher positive rate of T-SPOT®.COVID than those not hospitalized. A previous study reported that early nasal mucosal immune response as a type of natural immunity was higher in children than in adults, which was inversely correlated with the severity of COVID-19 [13]. Therefore, the nasal mucosal immune response may be heightened, potentially reducing the need for robust cellular immunity in children who did not require hospitalization.

This study presented some limitations. First, the sample size was small. Regrettably, collecting samples for cellular immunity studies in children poses a challenge, as more than 5 mL of blood is required, necessitating immediate separation of lymphoid cells. Hence, our cellular immunity samples from children are considered valuable. Second, cellular immunity is generally considered to be immature in childhood [14], which might affect the responsiveness of the T-SPOT®.COVID test in children. Notably, interferon-gamma release assays, including the T-SPOT® test, for diagnosing latent tuberculosis, have uncertain efficacy in children owing to the immaturity of cellular immunity [15]. Nevertheless, in our study, the T-SPOT®.COVID test results were positive among children post-COVID-19 vaccination, suggesting that this kit may be effective for use in children. Third, we could not follow participants for more than 6 months, which is not a long time. The level of SARS-CoV-2-specific cell immunity is reported to persist for more than half a year following COVID-19 vaccinations in adults. Additionally, the level of SARS-CoV-2-specific cell immunity is also noted to continue for at least 1 year after contracting COVID-19 in adults. Therefore, it is imperative to monitor children following COVID-19 vaccinations or diagnosis for more than half a year into the future.

5. Conclusions

Our study investigated cellular immunity for COVID-19 in children using T-SPOT®.COVID. Our findings affirmed the utility of this kit in assessing cellular immunity in pediatric populations. We observed that children can develop cellular immunity against COVID-19 through vaccination; however, acquisition of immunity may be limited in those affected by COVID-19, particularly if their illness is not severe. Moving forward, we anticipate that future studies will provide further insights into cellular immunity for COVID-19 among children, contributing to a deeper understanding of this important aspect of pediatric health.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author, Tomohiro Oishi, upon reasonable request.

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