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Toll-like Receptor 2 Signaling Abnormalities Are Associated with Clinical Manifestations in Common Variable Immunodeficiency

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ABSTRACT

Background: Common Variable Immunodeficiency (CVID) is an inborn error of immunity characterized by a defect in terminal B cell differentiation, resulting in hypogammaglobulinemia, and impaired production of specific antibodies. Stimulation via Toll-like receptors (TLRs) has been shown to promote the differentiation and functional maturation of late-stage B cells.

Objective: To assess aberrations in TLR2 signaling among patients with CVID and to explore their associations with clinical manifestations and immunological parameters.

Methods: Sixteen CVID patients and 16 healthy controls were recruited for this individual-matched case-control study Genetic variants in patients had been previously identified through whole-exome sequencing. TLR2 and TLR4 downstream gene expression were analyzed using qRT-PCR, while cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA). Statistical associations between clinical features and laboratory parameters were analyzed using SPSS software.

Results: downstream gene expression following TLR2 stimulation was significantly reduced in 25% of CVID patients, while the TLR4 signaling pathway remained largely unaffected. Patients exhibiting TLR2 overexpression demonstrated a later disease onset, presenting with autoimmunity, lymphoproliferation, and atopic manifestations. A consistent immunologic feature among patients with defective TLR2 signaling was the reduction in marginal zone and switched memory B cell populations. Furthermore, Levels of IL-6 and IL-1β following agonist stimulation were significantly lower in CVID patients compared to healthy controls.

Conclusion: This study demonstrates that functional impairment of TLR2 signaling influences the clinical presentation, immunologic profile, and cytokine production in patients with CVID. These findings suggest a potential underlying etiology in a subset of patients with unidentified monogenic defects.

Keywords: Primary immunodeficiency, Inborn errors of immunity, Common Variable Immunodeficiency, Toll-like receptors, Cytokine production

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INTRODUCTION

Common Variable Immunodeficiency (CVID) is the most common symptomatic form of inborn errors of immunity (IEI) or primary immunodeficiencies. It is characterized by hypogammaglobulinemia and impaired production of specific antibodies against both polysaccharide and protein antigens (1, 2). Patients with CVID are predisposed to recurrent sinopulmonary infections, autoimmune manifestations, lymphoproliferative disorders and an increased risk of malignancies (3-5).

Toll-like receptors (TLRs) are well-known members of pattern recognition receptors, expressed by both innate and adaptive immune cells, including B lymphocytes. TLRs recognize pathogen-associated molecular patterns (PAMPs) and host-derived damage-associated molecular patterns (DAMPs) (6). Their pivotal role in promoting antigen-independent antibody production-driving marginal zone and transitional B-cells toward plasma cell differentiation- and enhancing antigen-dependent responses-facilitating the maturation of follicular B-cells into memory and plasma cells-has been well established (7-10). TLR activation in B-cells augments proliferation, antibody and cytokine production, upregulation of co-stimulatory molecules, antigen presentation and immunoglobulin (Ig) class switching. Notably, functional impairments in TLR signaling have been reported in a subset of patients with CVID (11-14).

To date,, research on TLRs in CVID has predominantly focused on endosomal TLRs (11-16), with limited comprehensive data available on plasma membrane-associated TLRs in these patients. This study builds upon our previously published findings from 2018 and 2019 (17, 18), which characterized the baseline signaling profiles of TLR2 and TLR4, two surface-expressed TLRs involved in B-cell response to bacterial pathogens in CVID. In the present work, we extend these investigations through complementary experiments and reanalysis of existing data to deepen our understanding of TLR2 signaling abnormalities and their clinical implications. Furthermore, we delineate the correlations between clinical and immunological phenotypes and alterations in TLR2 and TLR4 signaling pathways.

MATERIAL AND METHODS

Study Population

Patients diagnosed with CVID patients and registered at the Clinic of Immunodeficiency, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran were enrolled in this study (19, 20). Inclusion criteria comprised a definitive diagnosis of CVID according to the European Society for Immunodeficiencies (ESID) guidelines (https://esid.org/Education/Diagnostic-Criteria-PID) (21), regular administration of intravenous Ig (IVIg), and absence of ongoing immunosuppressive therapy. Patients with a provisional CVID diagnosis and a confirmed pathogenic mutation in any of the 453 known monogenic IEI genes-based on the latest report from the International Union of Immunological Societies (IUIS) (22, 23)-were excluded following whole-exome sequencing analysis (19, 24, 25). Patients who had received IVIg treatment for less than 3 weeks, or who had a record of infection within the last 3 months were also excluded from the study. This was due to the potential impact on TLR pathways as suggested previously (26-30). This individual-matched case-control study included 16 CVID patients, each matched with a healthy control of the same age and sex. This study was approved by the Ethics Committee of Tehran University of Medical Sciences (Code: IR.TUMS.REC.1392.38734), and written informed consent was obtained from all participants prior to sample collection.

Clinical and Immunological Evaluation

An evaluation sheet was utilized to collect demographic data from CVID patients, including gender, ethnicity, place and date

of birth, medical history (with emphasis on the date of CVID diagnosis and comorbid conditions), clinical manifestations, and results from relevant genetic, immunological and laboratory assessments. Genetic variants had been previously identified through whole-exome sequencing. Data on TLR expression, specific antibody responses to Pneumovax-23 vaccination and B-cell subset distribution—including naïve B-cell, transitional B-cell, marginal zone-like B-cell, total memory B-cell, switched memory B-cell, IgM-only memory B-cells, plasmablasts and the subset of $CD21^{low}$ B-cells-were retrieved from our previous works (17, 18, 31, 32).

TLR Stimulation and Inhibition

Peripheral blood samples were collected immediately before IVIg infusion using ethylenediaminetetraacetic acid (EDTA)-containing tubes. Peripheral blood mononuclear cells (PBMCs) were isolated from patient and control samples via density gradient centrifugation using Ficoll-Paque (Biosera, France), followed by a single wash with phosphate-buffered saline (PBS). Cell viability was assessed by trypan blue exclusion. Cells were resuspended in complete culture medium composed of Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biosera, France) , 100U/mL penicillin (Dena Zist Asia, Iran), 100 µg/mL streptomycin (Dena Zist Asia, Iran), and 2 mM L-glutamine (Dena Zist Asia, Iran). For stimulation assays, 1×10^6 cells were cultured for 24 hours in the presence or absence of the following reagents: 1 µg/mL lipopolysaccharide (LPS; TLR4 ligand, Sigma-Aldrich, USA), 10 µg/mL lipoteichoic acid (LTA; TLR2 ligand, Sigma-Aldrich, USA), and 25 µg/mL oxidized phospholipid OxPAPC (TLR2/ TLR4 inhibitor, Invivogen, USA), previously described (17).

TLR Downstream Signaling Activation

Total RNA was extracted from cultured cells using Qiazole (Qiagen, USA) and reversed-transcribed into cDNA using the Takara cDNA synthesis kit (Takara, Japan) following the manufacturers' instructions. The expression levels of *RELA* (encoding nuclear factor kappa-light-chain-enhancer of activated B cells subunit 3; NF-κB3), *MAPK14* (encoding mitogen-activated protein kinase P38 alpha; P38), and *NFKBI* (encoding inhibitor of NF-κB alpha; IκB) were measured by quantitative real-time PCR using SYBR Green PCR Master Mix (Takara, Japan) with gene-specific primers (Table 1). Expression data were normalized to β-actin as the internal control.

Cell culture supernatants were collected 24 hours post-treatments and stored at -70°C until analysis. Concentrations of IL-1β and IL-6 were measured using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, USA) according to the manufacturer's protocol. The detection sensitivity for both cytokines was 2ρg/ml.

Statistical Analysis

Data were analyzed using SPSS software version 16. The Shapiro-Wilk test was applied to assess the normality of data distribution. Based on the outcome, appropriate parametric or non-parametric statistical tests were employed. Paired comparisons were conducted to evaluate differences in the expression of signaling element and cytokine production before and after stimulation assays. A p-values$\leq$0.05 was considered statistically significant.

Table 1. Oligonucleotide primer sequences used for quantitative real-time PCR analysis

Genes	Forward	Reverse
ACTB	F; 5'-GTGGGGCGCCCCAGGCACCA-3',	R; 3'-CTCCTTAATGTCACGCACGATTTC-5'
NFKBI	F; 5'-CTCCACTCCATCCTGAAGGCTA-3',	R; 3'-AGGTCCACTGCGAGGTGAAG-5'
MAPK14	F; 5'-GAGGTGCCCGAGCGTTAC-3',	R; 3'-GGAGAGCTTCTTCACTGCCAC-5'
RELA	F; 5'-GCTACACAGGACCAGGGACAGT,	R; 3'-AGCTCAGCCTCATAGAAGCCATC-5'

RESULTS

Sixteen adult patients diagnosed with CVID were enrolled in this study, comprising 10 (62.5%) males and 6 (37.5%) females with a mean age of 26.4±9.0 years. The majority (13 patients, 81.2%) had disease onset during childhood. Phenotypic classification revealed infections-only in 3, autoimmunity in 9, lymphoproliferation in 5, allergy in 4 and enteropathy in 4 patients. No cases of malignancy were observed. Detailed Demographic and immunological characteristics of the study cohort are presented in Table 2.

Increased TLR2 Expression with Preserved TLR4 Expression in CVID Patients

TLR2 expression was significantly elevated in CVID patients compared to healthy controls (17.9±9.7% *vs.* 12.7±5.2%, p=0.049). Notably, CVID patients with autoimmune manifestations exhibited slightly higher TLR2 levels (19.5±11.8%) than those without autoimmunity (14.4±5.5%). This upregulation was particularly pronounced in patients with autoimmune cytopenia relative to those with other autoimmune conditions (21.3±15.7% *vs.*17.8±7.8%, p=0.03). Elevated TLR2 expression was also observed in patients with atopic diseases (19.8±6.7% *vs.* 17.3±10.7%) and lymphoproliferative disorders (20.0±13.1% *vs.* 16.2±6.5%) when compared to CVID patients without these complications. In contrast, patients with an infection-only phenotype demonstrated significantly lower levels of TLR2 (11.1±2.4%) compared to those with other clinical phenotypes (19.5±10.1%, p=0.01). In contrast, individuals with

Baseline TLR4 expression did not differ significantly between CVID patients and healthy controls (14.7±4.2% *vs.* 14.1±7.6%, p=0.34). Furthermore, LPS stimulation and subsequent cytokine profiling confirmed intact TLR4 signaling in CVID patients. Tables 3 and 4 summarize the TLR2 and TLR4 signaling profiles, respectively, in CVID patients compared to healthy individuals.

Table 2. Clinical and immunological characteristics of CVID patients

Subjects	Total patients	Patients with TLR2 defect	Patients with normal TLR2
Number of patients	16	4	12
Sex (male/female)	10/6	3/1	7/5
Age; year (years, mean±SD)	26.4(9.0)	30.7(8.2)	24.4(11.6)
Age of onset (years, mean±SD)	14.5±9.2	14.7±13.7	7.7±7.2
Diagnosis delay (years, mean±SD)	5.0±3.8	8.3±6.2	4.3±2.7
Consanguinity (%)	11(68.7)	3(75)	8(66.6)
Infection only phenotype (%)	2(12.5)	0	2(16.6)
Autoimmunity (%)	9(56.2)	4(100)	5(41.6)
Enteropathy (%)	4(25)	1(25)	3(25)
Lymphoproliferation (%)	5(31.2)	4(100)	1(8.3)
Allergy (%)	4(25)	3(75)	1(8.3)
IgG (mg/dl, mean±SD)	234.1±222.7	263.7±166.7	166.4±107.1
IgA (mg/dl, mean±SD)	25.3±24.7	16.0±13.8	29.2±24.5
IgM (mg/dl, mean±SD)	36.1±45.4	34.7±25.3	33.7±25.0
Leukocytes (cell/ul, mean±SD)	8878.7±3254.0	7367.5±3230.2	9382.5±3238.2
Lymphocytes (% of leukocytes, mean±SD)	30.2(21.5)	35.3(15.9)	28.3(12.6)
$CD3^+$ (% of lymphocytes, mean±SD)	79.0(9.7)	80.6(11.9)	78.6(8.9)
$CD4^+$ (% of lymphocytes, mean±SD)	32.9(13.8)	23.8(9.6)	35.9(13.3)
$CD8^+$ (% of lymphocytes, mean±SD)	56.4(56.0)	42.9(23.2)	58.3(42.2)
$CD19^+$ (% of lymphocytes, mean±SD)	9.0(6.6)	4.9(3.9)	10.2(6.3)

Table 3. TLR2 signaling Profile in CVID patients compared to age- and sex-matched healthy controls

Parameters	Before TLR ligation CVID	After TLR ligation CVID	Before TLR ligation Controls	After TLR ligation Controls
TLR2 expression	17.9±9.7	15.5±6.2	12.7±5.2	9.2±4.8
NFKBI expression	10.3±4.8	3.0±2. 7	8.6±7.0	0.4±0.3
MAPK14 expression	5.0±3.8	4.2±3.4	26.6±11.3	0.21±0.19
RELA expression	13.7±11.7	16.2±12.0	11.4±8.8	1.27±1.04
IL6 production	2820.8±825.8	14037±13947.5	9760.0±2389.4	21486.0±15310.1
IL-1β production	163.2±39.7	1533.5±1546.1	791.9±234.2	3445.9±302.1

Table 4. TLR 4 signaling profile in CVID patients compared to age- and sex-matched healthy controls

Parameters	Before TLR ligation CVID	After TLR ligation CVID	Before TLR ligation Controls	After TLR ligation Controls
TLR4 expression	14.7±4.2	17.8±9.8	14.1±7.6	10.4±5.2
NFKBI expression	10.3±4.8	14.4±1.4	8.6±7.0	84.0±15.1
MAPK14 expression	5.0±3.8	23.3±12.6	26.6±11.3	2.1±1.3
RELA Expression	13.71±11.7	9.66±9.47	11.4±8.8	20.9±17.8
IL6 production	2820.8±825.8	16219.0±15196.9	9760.0±2389.4	23608±16599.8
IL-1β production	163.2±39.7	1050.5±602.7	791.9±234.2	2103.9±1579.3

Overlapping Clinical Phenotypes in CVID Patients with TLR2 Signaling Abnormalities

Using a threshold defined as >2 standard deviations above the mean TLR2 expression in healthy individuals,, a subset of CVID patients (N=4, 25%) was identified with markedly elevated TLR2 levels. In contrast, no outlier CVID patients were observed based on TLR4 expression relative to .of healthy controls. All four patients with TLR2 overexpression exhibited autoimmune cytopenia and lymphoproliferation disorders, while atopic manifestations were documented in three of them.

Previously published data on this cohort reported reduced frequencies of marginal zone and switched memory B cells, which support the current findings (31). Although total lymphocyte counts did not differ significantly among CVID patients, B-cell subset analysis revealed a distinct immunophenotypic profile in the TLR2-overexpressing group. Specifically, these patients showed a trend toward lower percentage of total B-cells (4.9±3.9% *vs.* 10.2±6.3%; p=0.11), with significantly reduced marginal zone B cells (0.26±0.1% *vs.* 5.7±4.9%, p=0.02) and switched memory B cells (0.1±0.05% *vs.* 0.5±0.4%, p=0.02). An increased proportion of $CD21^{low}$ B cells was also observed in this group (26.6±15.9% *vs.* 20.7±19.9%, p=0.6), although the difference was not statistically significant.

Re-analysis of whole-exome sequencing data did not reveal any deleterious variant in the TLR genes or in genes associated with the TLR2 signaling pathway-including *CD14, CLEC7, CYBB, CXCR4, IRAK3, IRAK4, LY96, MYD88, TOLLIP, TIRAP, SIGIRR, SAA1, SFTPA1, NFKBI, MAPK14* and *RELA*- in these 4 patients (Table 5).

Impaired TLR2 Downstream Signaling and Cytokine Production in CVID Patients

In the subgroup of CVID patients with elevated TLR2expression, stimulation with LTA led to a significant reduction in TLR2 levels across all patients. The magnitude of TLR downregulation in these patients (9.7±2.4%) was significantly greater than that observed in other CVID patients (-0.03±4.2%, p=0.02) and healthy individuals (3.2±2.8%, p=0.04). Treatment of PBMCs from CVID

Table 5. Genetic variants in TLR2-associated genes identified in 4 CVID patients with TLR2 overexpression

Genes	Patient 1	Patient 2	Patient 3	Patient 4
CD14	-	-	-	-
CLEC7A	Het c.492+161C>A	-	-	-
CYBB	-	-	-	-
CXCR4	-	-	-	-
IRAK3	-	-	Het c.256A>G p.I86V	Het c.256A>G p.I86V
IRAK4	-	-	Het c.800G>A p.R267H	-
LY96	-	-	Het c.166A>G p.R56G	Het c.166A>G p.R56G
MYD88	Het c.367+223C>A	-		-
TOLLIP	-	Het c.610+2525delC	Het c. c.664G>T p.A222S	-
TIRAP	-	-		Het c. 539C>T p.S180L
SIGIRR	-	-	Het c.935A>G p.Q312R	Het c.239C>A p.S80Y
SAA1	-	-	Het c.228+2C>G	Het c.209C>T p.A70V
SFTPA1	-	-	-	Het c.56T>C p.V19A
NFKBIA	-	Het c.1985G>A, p.R662E	-	-
MAPK14	-	-	-	-
RELA	-	-	-	-
TLR1	-	Het c.194T>C, p.I65T	-	Het c.239G>C p.R80T
TLR2	-	-	-	-
TLR3	Het c.634-10C>A	-	Het c.1234C>T p.L412F	-
TLR4	-	-	-	-
TLR5	-	-	Het c. 2464T>C p.F822L	-
TLR6	-	-	-	Het c.745T>C p.S249P
TLR7	-	-	Het c.32A>T p.Q11L	Het c.32A>T p.Q11L
TLR8	-	-	Het c. 1A>G p.M1V	-
TLR9	-	-	-	-
TLR10	-	-	-	Het c.2323A>G p.I775V

patients with TLR2 overexpression with TLR2/4 inhibitor (OxPAPC) resulted in a substantial decrease in TLR2 expression (from 29.8±11.5% to 12.5±9.4%; difference of -17.1±7.3%). In contrast, OxPAPC treatment led to a slight increase in TLR2 expression in other CVID patients (from 13.9±4.8% to 14.6±3.6%; difference of 1.3±0.9%; p=0.001) and in healthy controls (from12.7±5.2% to 15.6±7.0%; difference of 2.8±2.0; p<0.001).

mRNA expression levels of *NFKBI*, *MAPK14* and *RELA* genes encoding NF-κB3, IκB and p38 proteins, respectively, were assessed following TLR2 ligation

with LTA. Although a 3- to 5-fold change in gene expression was observed in TLR2-overexpressing patients compared to other CVID patients and healthy individuals, these differences did not reach statistical significance. Cytokine assay revealed significantly reduced baseline IL-6 production in CVID patients compared to healthy controls (2820.8±825.8 ρg *vs.* 9760.0±2389.4 ρg, p=0.015). LTA stimulation partially restored IL-6 levels (14037.0±13947.5 *vs.* 21486.0±15310.18 ρg, p=0.25), but, this compensatory response was absent in the TLR2-overexpressing subgroup (3499.0±2825.8 ρg) versus patients with normal TLR2 expression (14216.6±13145.2 ρg, p=0.02).

Similarly, IL-1β secretion was significantly lower in CVID patients compared to healthy individuals (163.2±39.7 ρg vs. 791.9±234.2 ρg, p=0.019). LTA stimulation enhanced IL-1β production (1533.5±1526.1 ρg vs. 3445.9±302.1, p=0.031), yet patients with TLR2 overexpression showed a blunted response (363.5±158.9 ρg) compared to other CVID patients (1580.0±1547.5 ρg, p=0.04).

Defective specific antibody production in CVID patients with TLR2 Overexpression

A significant inverse correlation was observed between TLR2 expression and IgG response to Pneumovax-23 vaccination ($r=-0.71$, p=0.03), while IL-6 levels following LTA stimulation showed a strong positive association with IgG response ($r=0.89$, p=0.01) in CVID patients. All patients exhibiting TLR2 overexpression and impaired TLR2 signaling were classified as hypo-responders to the vaccine. Their mean IgG levels post-vaccination (8.6±15.7 IU/ml) were significantly lower than those of CVID patients with normal TLR2 expression (76.1±63.1, p=0.049).

DISCUSSION

CVID represents a diagnostic category within inborn errors of immunity (IEI), characterized by heterogeneous clinical manifestations (4, 5). Although genetic defects are implicated in its pathogenesis, the precise etiology remains unknown in the majority of cases. Dysregulation of TLR signaling has been suggested to contribute CVID pathogenesis; however, the full extent of its clinical relevance has not been well studied (33). In this study, we focused on two key TLRs—TLR2 and TLR4— due to their established roles in bacterial recognition and antibody production. We evaluated their signaling dynamics, alongside downstream cytokine responses and specific antibody production, using targeted agonists and inhibitory compounds.

In this study, we identified approximately 25% of CVID patients without known monogenic defects who exhibited a marked impairment in TLR2 signaling. TLR2 functions not only as a cell-surface receptor expressed by a wide range of human cells but also in soluble secreted forms, enabling the detection of both endogenous DAMPs and exogenous PAMPs (34). TLR2, either in homodimeric or as a heterodimer with TLR1 and TLR6, recognizes a broad spectrum of microbial components, including lipoteichoic acid, bacterial peptidoglycan, bacterial triacylated lipopeptides, viral hemagglutinin, and diverse glycoproteins. Engagement of TLR2 initiates pro-inflammatory signaling through MAPK and MYD88-dependent pathways, while simultaneously activating anti-inflammatory responses via PI3K/AKT-dependent mechanisms (35). The resulting inflammatory signals drive NF-κB activation and nuclear translocation, thereby regulating transcription of genes encoding inflammatory cytokines such as IL-1β and IL-6. Furthermore, TLR2 is upregulated on B cells upon activation in secondary lymphoid organs and plays a critical role in shaping antigen-specific IgG responses (36).

Although most CVID patients present with a single phenotype, predominantly characterized by recurrent infections (37), the subset of patients in our study with TLR2

overexpression displayed an overlapping phenotype that included autoimmunity, lymphoproliferation and atopic disorders, alongside a later disease onset. . Notably, the infection-only phenotype showed an inverse association with TLR2 expression levels in our CVID patients. Previous studies have linked autoimmune and lymphoproliferative diseases in CVID to T cell hyperactivity (38, 39) and B cell overstimulation via the TLR2 pathway, resulting in excessive autoantibody production and increased inflammatory cytokine release (35, 40). Importantly, a significant reduction in marginal zone B cells has been reported as a consequence of TLR2-associated inflammatory disorders (41), a finding consistent with the immunological phenotype in the subgroup of TLR2-defective CVID patients in the present study.

Atopic manifestations are uncommon complications in CVID patients, yet they were observed at a higher frequency among patients with TLR2 overexpression. Although limited, existing studies have suggested a role for TLR2 stimulation in promoting Th2-driven responses and hyper-reactive airway diseases (42, 43). However, the role of TLR2 in allergic responses is complex and appears to be influenced by the nature, dose, and type of TLR-associated ligands (44). Consistent with this complexity, we observed a significant reduction in IL-6 and IL-1β levels was documented in this subgroup of CVID patients, despite the fact that their TLR2 responded adequately to its cognate ligands. Furthermore, our data revealed a significantly diminished specific antibody response to Pneumovax-23 in CVID patients with TLR2 signaling defects. These findings are in agreement with those of Hong et al. (45); who demonstrated significantly reduced IL-6 production following TLR2 stimulation with Pneumovax-23in CVID patients compared with healthy controls. They concluded that IL-6 deficiency may contribute to increased susceptibility to *Streptococcus pneumonia* infection in CVID (45).

The identification of a subgroup of CVID patients with a prominent TLR2 overexpression adds to the growing body of evidence on diverse TLR impairments in CVID (11-14). Cunningham-Rundles was the first to demonstrate that, following TLR9 engagement with and without B-cell receptor (BCR) stimulation, CVID B-cells failed to upregulate CD86 indicating defective B-cell activation. Additionally, IL-6 and IL-10 production, as well as both surface and intracellular expression of TLR9 were impaired in CVID B-cells (11). These findings have since been supported by reports of defective TLR7 and TLR9 signaling in B-cells and plasmacytoid dendritic cells of CVID patients (12, 13). Importantly, TLR defects in CVID appear to be restricted and, in many cases, can be at least partially compensated by stimulation that restores cytokine production or normalizes circulating lymphocyte subsets (46). Consequently, therapeutic strategies targeting TLR ligands, adaptor proteins and downstream kinases, or negative regulatory molecules may offer promising adjuvant immunomodulatory approaches, particularly for a subgroup of CVID patients with an increased frequency of inflammatory complications.

The role of TLR4 in the pathogenesis of CVID remains completely understood. In the present study, we did not observe any significant differences in TLR4 expression or its downstream signaling when compared with healthy individuals. Nevertheless, TLR4 signaling defects could still represent a potential pathogenic mechanism in CVID, given the pivotal role of TLR4 in B-cell activation and antibody production through BCR-dependent SYK activation (47). Further investigations in larger, well-characterized cohorts of non-monogenic CVID patients will be essential to clarify the contribution of TLR4 dysregulation to CVID pathogenesis.

CONCLUSION

Collectively, our findings reveal functional

deficiencies in TLR2 downstream signaling in subset of CVID patients, marked by impaired production of IL-6 and IL-1β. Although ligand stimulation of TLR2 partially enhanced cytokine responses in CVID patients, it remained insufficient to restore cytokine production to physiologic levels. Our findings underscore the significance of TLR2 signaling abnormalities in CVID pathogenesis and support the need for stratified diagnostic and therapeutic approaches.

ABBREVIATION

CVID, Common variable immunodeficiency; TLR2, Toll-like receptor; IgM, Immunoglobulin M; IgG, Immunoglobulin G; IgA, Immunoglobulin A; NFKBI, Nuclear factor kappa B; MAPK14, Mitogen-Activated Protein Kinase 14; RELA, REL-associated protein; IL6, Interleukin-6; IL-1β, Interleukin-1 beta.

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AUTHORS' CONTRIBUTION

Hassan Abolhassani: Investigation, Methodology and Writing: Original Draft,. Nima Rezaei: Conceptualization, Resources and Writing: Review & Editing. Reza Yazdani: Investigation, Formal analysis, and Software support. Somaye Aletaha: Methodology and Writing: Review & Editing. Saied Bokaie: Investigation and Formal analysis. Laleh Sharifi: Project management, Supervision, Investigation, Methodology, Formal analysis, Writing: Original Draft. Abbas Mirshafiey: Project administration, Supervision, Validation, and provision of Resources. All authors reviewed and approved the final manuscript.

AVAILABILITY OF DATA

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Due to privacy and ethical considerations, the data are not publicly accessible.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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