

Original article

Defective T-cell activation caused by impairment of the TNF receptor 2 costimulatory pathway in common variable immunodeficiency

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Background: Patients with common variable immunodeficiency have defective T-cell activation after stimulation via T-cell receptor (TCR)/CD28 or by recall antigens.

Objective: In the current study, we investigated whether TNF-receptor 2 (RII) costimulation, which is important for sufficient TCR/CD28 stimulation, was significantly impaired in common variable immunodeficiency (CVID).

Methods: We studied T-cell activation events such as CD69 induction, calcium flux through store operated calcium channels, protein kinase C- θ translocation, and costimulation via TNF-RII compared with costimulation via CD28.

Results: By measuring TNF receptor-associated factor 1 expression, which is induced by TCR alone and can be upregulated by either CD28 or TNF-RII costimulation, we show that costimulation via CD28 is intact, whereas costimulation via TNF-RII in these patients is impaired. The ras-activation pathway as tested by CD69 induction, calcium flux through store operated calcium channels, and protein kinase C- θ translocation were comparable in CVID and control T cells.

Conclusion: Taken together, these data indicate that the primary TCR signal as well as the signal derived from CD28 are normal but that TNF-RII-supported TCR costimulation is defective, most likely leading to impairment of an important amplification loop, such as TNF-RII augmented nuclear factor- κ B activation.

Clinical implications: The finding of defective TNF-RII cosignaling in patients with CVID may help to define the activation pathway affected, thus potentially leading to a characterization of the molecular defect and molecular diagnosis in at least some of these patients. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: CVID, primary immunodeficiency, T-cell receptor, TNF- α , TNF-RII, TRAF1, calcium flux, store operated calcium channels, PKC- θ

Abbreviations used

CVID: Common variable immunodeficiency

PKC: Protein kinase C

PMA: Phorbol 12-myristate 13-acetate

SOC: Store-operated calcium

TCR: T-cell receptor

TNF-RII: TNF-receptor 2 (p75, CD120b)

TRAF: TNF receptor-associated factor

Common variable immunodeficiency (CVID) is made up of a heterogeneous group of clinically severe primary antibody deficiency syndromes primarily characterized by a pronounced defect in the production of IgG antibodies of sufficient titers and affinity to a variety of antigens.¹ Serum levels of IgG and/or other immunoglobulin classes are low, and the patients present with increased susceptibility to infection. In the majority of patients, the underlying genetic defect is unknown. Defects in B-cell activation, such as via Toll-like receptor 9,² in T cells,³⁻⁵ in dendritic cells,⁶⁻⁸ and a deficiency in circulating natural killer cells, have been found.⁹ In addition, a marked restriction of the T-cell receptor (TCR) repertoire with an increased oligoclonal expansion of CD8⁺CD28⁻ cells has been reported in patients with CVID.¹⁰ Single patients with defects in signal transduction elements involved in TCR activation such as impaired coupling of ζ -associated protein 70 to the ζ -chain of the TCR/CD3 complex³ or induction of vav expression¹¹ have been described. Mutations in the inducible costimulator gene¹² and in the transmembrane activator and calcium-modulator and cytophilin-ligand interactor (TACI) gene^{13,14} have been identified in some patients, whereas in the majority of patients with CVID, these genes are intact.^{15,16}

Several intracellular pathways are involved in amplification and/or integration of the activation signal derived from the TCR plus a costimulatory molecule, such as the ras pathway,^{17,18} TCR-mediated activation of protein kinase C (PKC)- θ ,¹⁹⁻²¹ calcium flux through store operated calcium channels,²² and TNF-receptor 2 (RII)—mediated costimulation of T-cell activation.²³⁻²⁵ In this study, we further characterize defective TCR-dependent stimulation in patients with CVID by investigating these distinct signaling events and/or pathways.

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TABLE I. Patients' characteristics at time of diagnosis or study enrollment

Patient no.	Age (y)	Time since diagnosis (y)	Sex	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	Tetanus toxoid IgG (IU/mL)	Pneumococcal polysaccharide IgG (titer)	Hib IgG (μg/mL)
				At time of diagnosis or first consultation					
1	78	8	F	367	57	38	NA	1:82	<0.1
2	54	1	F	150	<6	231	0.42	1:20	0.72
3	70	15	F	248	18	141	NA	NA	NA
4	33	<1	M	84	<7	9	0.01	<1:20	0.01
5	60	5	F	414	10	105	NA	<1:20	0.05
6	43	14	F	105	0	58	NA	NA	NA
7	22	19	M	510*	<6	6	1.23*	1:380*	2.91*
8	61	14	F	32	0	0	NA	NA	NA
9	57	12	F	494	62	279	NA	<1:20	NA
10	25	3	M	148	8	6	0.08	<1:20	0.02
11	53	5	F	56	<7	8	0.02	<1:20	0.02
12	37	5	F	217	<8	33	0.08	1:45	0.89
13	25	<1	M	10	6	8	<0.01	<1:20	<0.01
14	66	9	F	199	<8	49	NA	1:63	0.2
15	39	6	F	239	<8	47	0.08	<1:20	<0.1
16	39	<1	M	288	<7	6	0.71	1:99	2.12
17	52	26	M	715*	<7	<6	NA	1:376*	0.57
18	56	48	F	69.4	243	354	NA	<1:20	NA
19	37	1	F	10	<7	<5	<0.01	<1:20	0.07
20	80	15	M	<7	0	0	NA	NA	NA
21	35	2	M	870*	<6	7	1.72*	1:581*	5.23*
22	70	8	F	206	<7	51	0.1	1:17	0.14
23	45	<1	F	202*	<7	90	0.01	1:20	0.05
Mean	49.4	9.9							
Normal range				815-1784	93-287	108-237	>0.4	>1:200	>1

Hib, Hemophilus influenzae type b polysaccharide; F, female; M, male; NA, not available.

*Under IVIG.

METHODS

Patients and controls

Twenty-three patients with CVID (Table I) diagnosed according to the classification of the International Union of Immunological Societies¹ on primary immunodeficiency diseases gave their informed consent to repeatedly donate blood for the study. Consecutive patients were included into the study if they had no signs of an acute infectious episode at the time of blood sampling. None of the patients had the clinical phenotype of granulomatous disease, and none of them was on steroids or on other anti-inflammatory medications during the study. Heparinized whole blood units from voluntary donors were obtained from the Austrian Red Cross (Vienna, Austria) and served as healthy controls. Of the 23 consecutive patients with CVID investigated, 14 patients were available for TNF-RII investigation (either for TNF-RII-induced TNF receptor-associated factor [TRAF]—1 expression or proliferation or both). Intravenous immunoglobulin (IVIG) substitution controls consisted of 2 patients with X-linked agammaglobulinemia (XLA) (patients 3 and 5²⁶), a patient with IgG subclass deficiency, and a patient with IgM deficiency receiving IVIG substitution therapy.

Cell separation, preculture, and restimulation

PBMCs were separated from whole blood by density gradient centrifugation (Lymphoprep; Invitrogen, Lofer, Austria). PBMCs

were depleted of monocytes by adherence to plastic, and nonadherent cells were precultured for 10 to 17 days either unstimulated or stimulated (anti-CD3 mAb, clone HIT3a; PharMingen Europe, Becton Dickinson, Schwench, Austria; 10 ng/mL unless stated otherwise) before restimulation with anti-TCR + anti-CD28 as previously described²⁷ (Fig 1).

Freshly isolated nonadherent lymphocytes or precultured lymphocytes (Fig 1, A) were stimulated with anti-TCR mAb (clone BMA 031; Immunotech, Marseille, France; 750 ng/10⁷ beads, 5 × 10⁵ beads/mL, coated onto Dynabeads M-450; Dynal, Oslo, Norway; according to the manufacturer's protocol). To deliver a cosignal, anti-CD28 mAb (clone CD28.2; Immunotech; 10 ng/mL) was used (Fig 1, A). Recombinant human TNF-α (R&D Systems, Minneapolis, Minn; 20 ng/mL) or anti-TNF-RII mAb (clone 22221.311; R&D Systems; 1 μg/mL) in combination with TCR-mAbs was used to test the costimulatory activity of the TNF-RII receptor (Fig 4, A-C). To assess the contribution of TNF-RII triggering via endogenous TNF-α, endogenous TNF-α was neutralized by anti-TNF-α (Upstate Biotechnology, Lake Placid, NY; clone 2CB, 1 μg/mL). In proliferation assays, cells were stimulated for 5 days; for IL-2 release and TRAF1 expression, cells were stimulated for 24 hours (Fig 4; Fig 5, A-C). In Fig 6, nonadherent lymphocytes were stimulated for 3 days either with phorbol myristate acetate (PMA; 10 ng/mL; Sigma-Aldrich, Vienna, Austria) plus ionomycin (500 ng/mL; Sigma-Aldrich), anti-TCR beads alone, or TCR plus CD28 or TNF-RII or the respective isotype controls (control beads). TRAF1

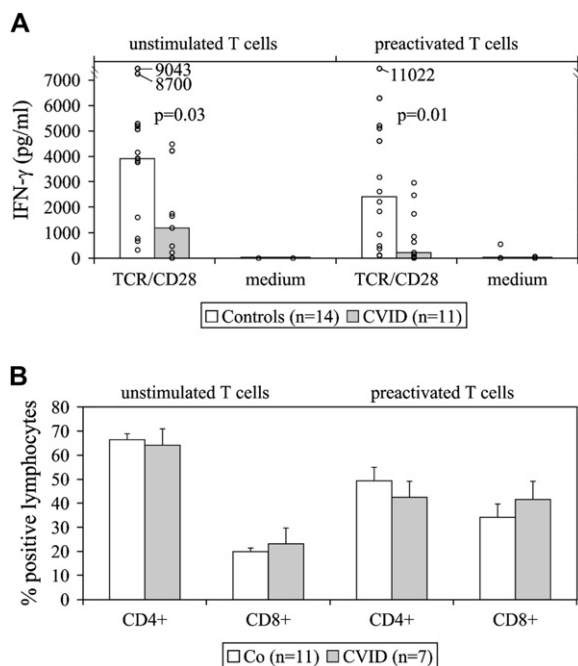


FIG 1. Defective TCR stimulation in patients with CVID is not corrected if T cells are preactivated. T cells were precultured with or without anti-CD3 mAb for 10 to 17 days and restimulated with anti-TCR/anti-CD28 mAbs for 5 days. IFN- γ release was determined by ELISA (**A**; bars = median). At the end of preculture, the percentage of CD4 and CD8 positive cells was determined by flow cytometry (**B**). Co, Controls.

and TRAF2 expression was investigated by Western blot. Fig 6, A, shows the OD readings (mean $\pm$ SEM) for patients and controls, Fig 6, B, shows the individual Western blots, and in Fig 6, C, the ratio of the OD readings for TCR/TNF-RII divided through the OD readings for TCR/CD28 was calculated.

In proliferation assays, cells were pulsed with ^3H -thymidine for the last 16 hours, and ^3H -thymidine incorporation was measured with a liquid scintillation counter (Wallac 1450 MicroBeta Trilux, Perkin Elmer Life Sciences, Vienna, Austria). Cytokines were measured with sandwich ELISA kits (IFN- γ , IL-2; Flexia; Biosource Europe SA, Fleurus, Belgium) according to the manufacturer's instructions. In Fig 1, A, IFN- γ secretion was investigated after preculture with or without stimulation with anti-CD3 mAbs for 10 to 14 days and subsequent stimulation for 5 days via TCR and CD28.

Western blot

In translocation assays, PBMCs were stimulated with PMA (10 ng/mL) for 15 minutes before membrane and cytosolic fractions were isolated as previously described.²⁸ Protein extracts from whole cell lysates or membrane and cytosolic fraction were subjected to SDS-PAGE electrophoresis and proteins transferred to a nitrocellulose membrane. For blotting, the following antibodies were used: PKC- θ (BD Biosciences, Erembodegem, Belgium; mouse IgG_{2a} directed against a 21-217-epitope) and phospho-PKC- θ (Cell Signaling Technology, Inc, Danvers, Mass; specific for phospho-Thr538), with both revealing similar results; PKC- βI (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif; clone C-16), fyn: rabbit anti-fyn antiserum (Upstate Biotechnology, specific for a 35-51 epitope), TRAF1 (Santa Cruz Biotechnology, Inc.; clone H-3, mouse IgG₁ against the 173-295 epitope), and TRAF2 (Santa Cruz Biotechnologies; clone H-249, rabbit IgG against the 1-249 epitope).

The proteins were detected using the SuperSignal West Pico ECL detection system (Pierce, Rockford, Ill) with subsequent densitometric quantification. The reproducibility of TRAF1 protein quantification using this method was 12.3% (ie, the difference between 2 independent determinations on 1 sample of activated cells).

Flow cytometry

The following directly (fluorescein isothiocyanate, phycoerythrin, and peridinin chlorophyll protein) conjugated mAbs were used in 3-color immunofluorescence staining following a standard protocol: CD3, CD4, CD8, and CD69 (all purchased from Becton Dickinson, Schwenhat, Austria). Cells were analyzed with a FACScan (Becton Dickinson) and CellQuest software (Becton Dickinson). In Fig 1, B, CD4 and CD8 subpopulations were determined in patients' and controls' nonadherent lymphocytes after preculture with or without CD3 stimulation before TCR + CD28 restimulation.

Ca⁺⁺ influx through store operated calcium channels

PBMCs ($2 \times 10^6/\text{mL}$) were stained for CD4 and loaded with Fluo-3 (Sigma-Aldrich; 1 $\mu\text{mol/L}$) and Snarf-1 (Molecular Probes, Eugene, Ore; 1 $\mu\text{mol/L}$) as described previously.^{27,29} Flow cytometry was performed in the presence of ethyleneglycol-bis(β -aminoethyl)-N,N,N',N'-tetraacetic acid (0.02 mmol/L; Merck, Vienna, Austria). After establishing the baseline calcium concentration, thapsigargin (500 nmol/L; Sigma-Aldrich) was added to deplete intracellular calcium stores. After 10 minutes, calcium chloride (0.07 mmol/L; Merck) was added to the medium, and calcium influx was measured. Analysis of fluorescence intensity and adjustment for control dye loading were performed with FlowJo software (Tree Star, Inc, Ashland, Ore).

Statistics

Results from repeated experiments with cells from different blood donors are given as mean of $n \pm$ SEM. Statistically significant differences between groups were calculated by using the Student t test or the Mann-Whitney U test as appropriate ($*P < .05$).

RESULTS

Preactivation of T cells does not correct impaired T-cell responsiveness in patients with CVID

Because preactivation lowers the threshold for T-cell activation in mature lymphocytes, we tested the effect of preactivation on TCR stimulation by preculture of T cells in the presence of a monoclonal anti-CD3 Ab and restimulation with anti-TCR/CD28-coated beads. Preactivation upregulated CD25, CD69, and HLA-DR expression (data not shown) and phenotypic investigation did not reveal differences in the proportions of CD4 and CD8 T cells between patients and controls (Fig 1, B). We found that the previously reported defective TCR-dependent T-cell activation (eg, impaired IFN- γ response) in CVID⁵ is not corrected by preactivation (Fig 1, A). IFN- γ release assessed in this system was broadly distributed in patients and controls; however, median values were significantly lower in the patients compared with controls ($P = .03$ and $.01$, respectively). This implies a substantial defect in at least 1 of the multiple pathways involved in

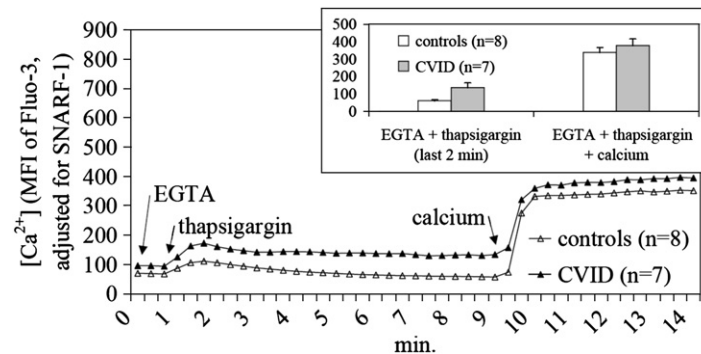


FIG 2. Calcium influx through SOC channels is normal in patients with CVID. PBMCs were loaded, stained for CD4, and resuspended in calcium-free medium. Baseline calcium concentration was established in the presence of ethyleneglycol-bis(β -aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA). Thapsigargin was added to deplete intracellular calcium stores. After a further 10 minutes, calcium was added, and the increase in intracellular calcium represents influx through SOC channels. *MFI*, Mean fluorescence intensity.

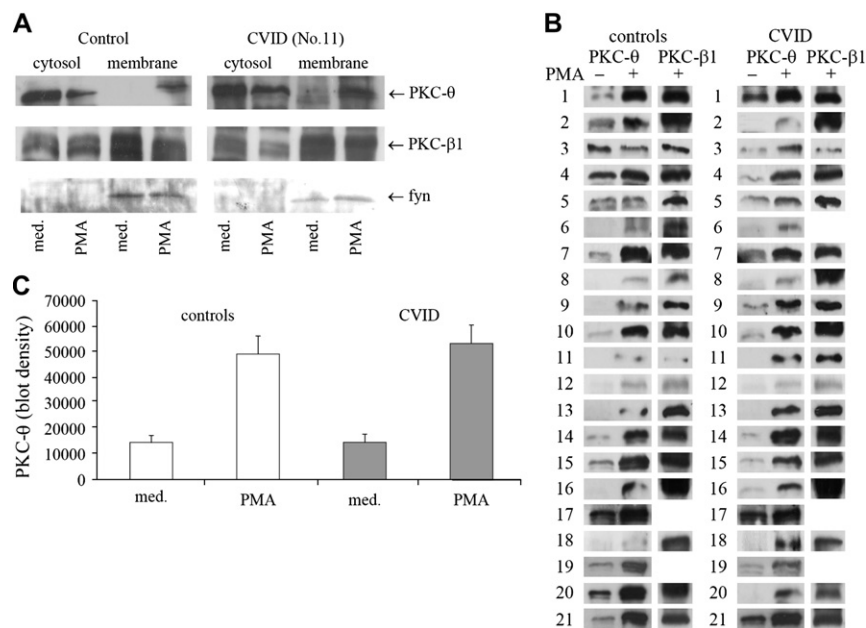


FIG 3. Translocation of PKC- θ is normal in patients with CVID. PBMCs were stimulated for 15 minutes (PMA 10 ng/mL), lysed, and separated into a membrane and cytosolic fraction. PKC- θ , PKC- β 1 (loading control), and fyn (membrane control) were examined by Western blot. (A) One representative experiment, (B) individual results for membrane extracts, and (C) densitometric readings (means $\pm$ SEMs) of membrane extracts. *med.*, Cells treated with culture medium alone.

T-cell activation in these patients and indicates that the underlying defect is not just a quantitative lack of sufficient costimulatory signals, because this should have been overcome by preactivation.

The ras-pathway is functional in patients with CVID

Although they use distinct signaling intermediates, both TCR-induced³⁰ and PMA-induced¹⁸ CD69 expression are dependent on ras activation. CD69 can be upregulated by TCR or PMA stimulation alone without the need for costimulation via CD28 or ionomycin,^{31,32} and TCR-induced

and PMA-induced CD69 expression were normal in patients with CVID (data not shown). These data indicate normal activation via the ras pathway and show for the first time that the primary TCR signal is not impaired in patients with CVID, at least as far as CD69 expression is concerned.

Calcium flux through store-operated calcium channels is normal in patients with CVID

To investigate calcium flux through store operated calcium channels, an important requirement for TCR-mediated T-cell activation,²² Fluo-3-loaded T cells were kept in calcium-free medium and stimulated with

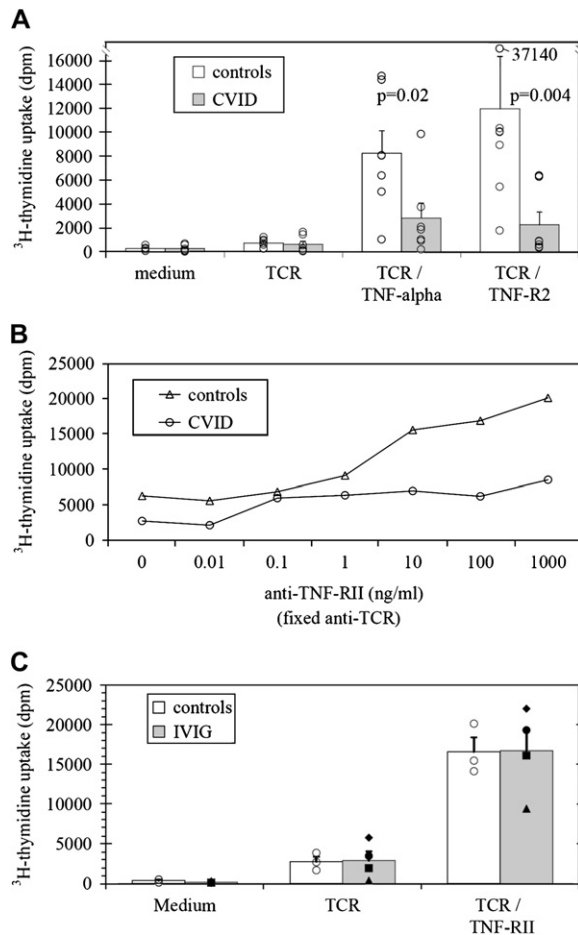


FIG 4. TCR costimulation via TNF-RII is defective in patients with CVID. Precultured (**A**; means $\pm$ SEMs; $n = 8$) or freshly isolated nonadherent lymphocytes (**B**; 1 experiment of 3) were stimulated with anti-TCR $\pm$ TNF- α or TNF-RII mAbs. **C**, Means $\pm$ SEMs, $\bullet$ = XLA patient 3, $\blacktriangle$ = XLA patient 5, $\blacksquare$ = IgG subclass deficiency, $\blacklozenge$ = IgM deficiency. dpm, Disintegrations per minute.

thapsigargin to deplete intracellular calcium stores and thereby open store-operated calcium (SOC) channels. Calcium was then added to the medium, and the resulting increase in intracellular calcium represents influx through SOC channels.³³ Patients with CVID had a normal increase in intracellular calcium in this system (Fig 2), indicating normal function of the store operated calcium channels, as measured in CD4-gated T cells. Thapsigargin-induced efflux from the intracellular calcium stores was increased in patients with CVID, the significance of which has to be determined (Fig 2).

Membrane translocation of PKC- θ is normal in patients with CVID

Stimulation of PBMCs with PMA for 15 minutes resulted in a marked shift of PKC- θ from the cytosolic fraction to the membrane fraction, as represented by a reduction in signal intensity in the cytosol (Fig 3, A) and a concomitant increase in signal intensity in the membrane fraction (Fig 3, A-C). As a membrane fraction-specific

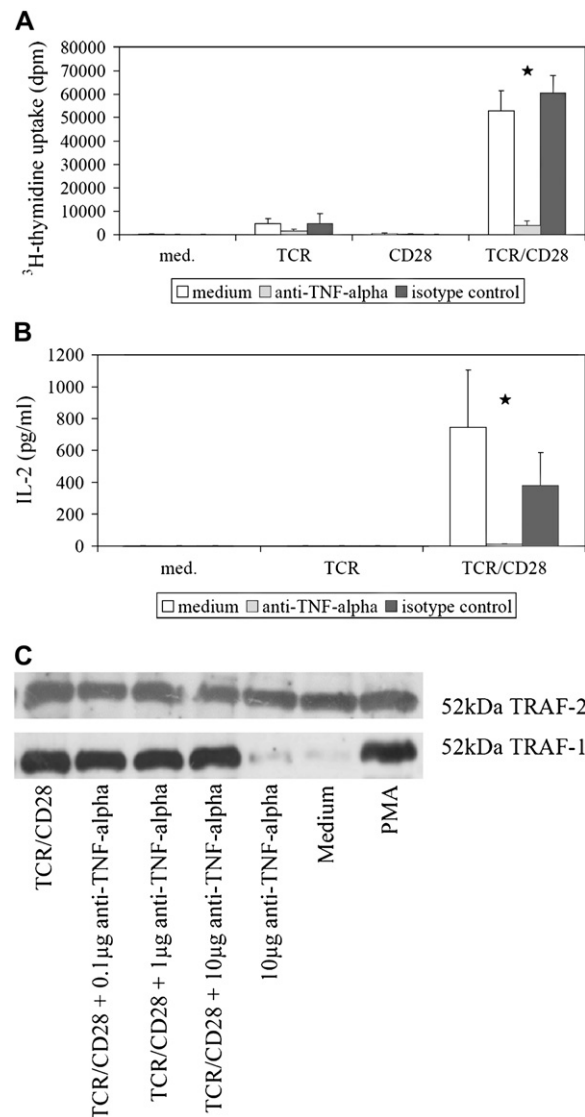


FIG 5. TCR/CD28-induced proliferation and IL-2 secretion but not TCR/CD28-induced TRAF1 expression requires concomitant TNF-RII triggering. T cells were stimulated with anti-TCR/CD28 mAbs in the presence or absence of anti-TNF- α or an isotype control mAb (1 $\mu\text{g}/\text{mL}$). Bars represent the means $\pm$ SEMs of 4 (**A**) or 3 (**B**; IL-2 release determined by ELISA) experiments. **C**, TRAF1: 1 representative Western blot experiment of 4. med., Cells treated with culture medium alone; dpm, disintegrations per minute.

control protein, we analyzed fyn in parallel (Fig 3, A), and as a control protein for protein loading, PKC- β 1 was examined (Fig 3, A and B). Densitometric readings of the individual Western blots in Fig 3, B, are shown in Fig 3, C. Patients with CVID and controls had comparable expression as well as comparable membrane translocation of PKC- θ .

The costimulatory effect of TNF-RII on TCR stimulation is defective in patients with CVID

Out of 23 consecutive patients with CVID included in this study, TNF-RII costimulation could be investigated in

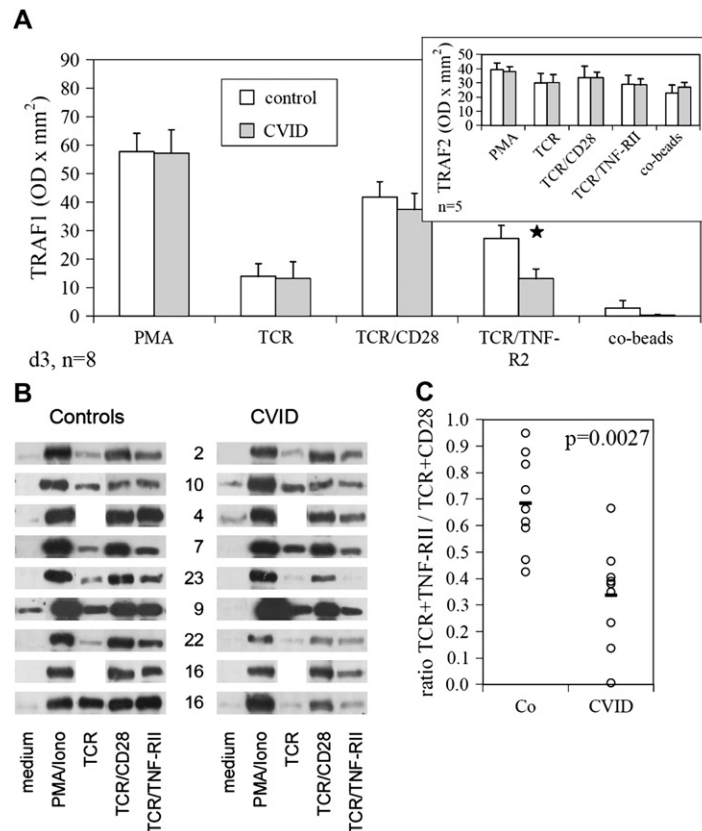


FIG 6. TCR and TCR/CD28-induced TRAF1 expression is normal in patients, but TCR/TNF-RII-induced TRAF1 expression is impaired. T cells were stimulated for 3 days (isotype control mAb coated beads = cobeads) and Western blots for TRAF1 (**B**) and TRAF2 were performed. The densitometric readings for TRAF1 are given in **A** (means $\pm$ SEMs; *insert* = densitometry for TRAF2). A ratio for TRAF1 densitometry was calculated in **C**. *Iono*, Lonomycin; *Co*, controls.

14 patients (in 2 patients both TNF-RII-mediated costimulation of proliferation and TRAF1 expression were examined). We found that not only TCR/CD28 but also TCR/TNF-RII induced proliferation is severely impaired in T cells of patients with CVID. Triggering of TNF-RII by TNF- α or by anti-TNF-RII mAbs gave comparable results (Fig 4, A). Defective costimulation via TNF-RII could also be confirmed by stimulating CVID T cells with increasing concentrations of TCR/TNF-RII mAbs (Fig 4, B), thus showing that reduced binding affinity of the mAb used is not a likely explanation. Moreover, TCR/TNF-RII costimulation was normal in 4 patients receiving IVIG substitution therapy (2 patients with XLA, 1 with IgG subclass deficiency, and 1 with IgM deficiency; Fig 4, C), showing that the defective TNF-RII costimulation was specific for patients with CVID and was not a result of IVIG substitution.

Neutralization of endogenous TNF- α during TCR/CD28 stimulation in the controls resulted in strong inhibition of T-cell proliferation (Fig 5, A) as well as IL-2 secretion (Fig 5, B) and IFN- γ release (data not shown), indicating that these important T-cell functions need the concomitant triggering of all 3 receptors. In contrast with proliferation and IL-2 secretion, TCR/CD28 induced

TRAF1 expression was not inhibited by neutralization of TNF- α (Fig 5, C), and TCR-induced TRAF1 expression was also enhanced by concomitant CD28 or TNF-RII triggering (Fig 6, A). As a loading control for TRAF1, TRAF2 was examined, which is constitutively expressed and not upregulated by T-cell activation (Fig 5, C). This indicates that each receptor alone is able to costimulate TRAF1 expression. Kinetic studies showed an almost parallel increase in TRAF1 for TCR, TCR/CD28, and TCR/TNF-RII for as long as 2 days, whereas the most pronounced costimulatory effect can be seen at day 3 when TCR-induced TRAF1 expression is coming back to baseline levels but TCR/CD28 and TCR/TNF-RII-induced TRAF1 expression remains high (data not shown).

Because TRAF1 expression can be independently enhanced by costimulation via CD28 or TNF-RII (Fig 5, C; Fig 6, A and B), TRAF1 expression is a suitable marker to distinguish between a defect in the CD28 versus the TNF-RII costimulatory pathway. CVID T cells had normal TRAF1 expression when stimulated either with TCR alone or with TCR/CD28, whereas stimulation of TRAF1 via TCR/TNF-RII was significantly impaired in the group of patients tested (Fig 6, A-C). In contrast, constitutive expression of TRAF2 was comparable in patients

and controls (Fig 6, A, *insert*). Two different experiments performed in patient 16 at 4 months apart revealed similar results (Fig 6, B), showing the stability of the patient's T-cell activation defect over time. The ratio of OD readings for TNF-RII-induced TRAF1 expression relative to that induced by TCR/CD28 is given in Fig 6, C. Calculation of this ratio confirms the significantly decreased TRAF1 induction by TNF-RII costimulation in patients with CVID.

DISCUSSION

Sufficient T-cell responses are obtained by repeated contact with the specific antigen or by nonspecific stimulation during development, priming, and conditioning of T cells, and preactivation lowers the threshold for T-cell activation in mature T cells up to several log units.^{34,35} Our finding that the patient's T cells do not improve TCR/CD28 responsiveness after preactivation with anti-CD3 indicates that not only the secondary responses but also the primary T-cell responses are impaired in these patients. Impaired TCR/CD28 responsiveness could not be explained by differences in CD4/CD8 distribution; however, the role of other subpopulations cannot be excluded at the moment.

Optimal T-cell responses require activation via at least 2 signals: the priming signal provided by the antigen receptor (TCR/CD3) complex plus a second signal provided by a costimulatory molecule. Previous studies in patients with CVID have indicated a receptor-proximal defect in TCR-dependent T-cell activation.⁵ A defect in proximal TCR signaling events such as defective ζ -chain phosphorylation³ or decreased induction of vav-expression¹¹ has been described in individual patients with CVID. Alternatively, a defect in 1 or more amplification mechanisms that is bypassed by direct PKC stimulation could lead to the partial T-cell activation defect seen in CVID.⁵

In the current study, we investigated proximal steps in TCR-dependent T-cell activation that are required for fully functional T-cell stimulation and had never been examined in CVID before. We show that SOC channel activity, which is required for prolongation of TCR-derived signals,²² is intact, as is membrane translocation of PKC- θ , a T-cell-specific signaling mechanism, and induction of CD69 expression, known to require activation of the ras-pathway.^{18,30} In contrast, our results show for the first time that TNF-RII-dependent costimulation of TCR activation is impaired in CVID. Because TNF-RII costimulation is indispensable for several important T-cell functions (Fig 5, A and B),^{23,24,36-39} a costimulatory defect of TNF-RII could be the underlying mechanism for the defective TCR/CD28 activation seen in these patients. Moreover, TNF-RII signaling is also required for CD40-induced IgM secretion in B cells, and TNF- α concentrations even 1000 times lower than required for modulation of TCR activation are effective.⁴⁰ Therefore, the observed defect in cell activation via TNF-RII could

contribute to defective TCR-mediated T-cell activation as well as defective antibody production in these patients.

Although CD28-mediated costimulation of proliferation and IL-2 and IFN- γ secretion is TNF- α -dependent, CD28-mediated upregulation of TRAF1 expression is not. CD28-mediated upregulation of TRAF1 protein was normal in patients with CVID, as was TCR-dependent TRAF1 induction, thus indicating that the primary TCR signal as well as the signal derived from CD28 are intact. However, TNF-RII induced costimulation of TRAF1 expression was impaired in CVID. The molecular mechanisms leading to impaired TNF-RII-mediated costimulation in CVID remain to be determined. We did not find defective TNF-RII expression in CVID (data not shown). Normal PMA-induced and TCR/CD28-induced TRAF1 expression almost certainly rules out a primary defect in TRAF1. TRAF1 induction undoubtedly involves nuclear factor- κ B activation,⁴¹ and an impairment of nuclear factor- κ B activation³⁹ and/or disturbed TRAF1-mediated regulation of TRAF2 activity⁴² could be involved in defective TNF-RII costimulation of CVID T cells.

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