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Toll-like receptor 7 and 9 defects in common variable immunodeficiency

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Abstract

Background—Common variable immunodeficiency (CVID) is characterized by hypogammaglobulinemia, reduced numbers of peripheral blood isotype-switched memory B cells, and loss of plasma cells.

Objective—Because Toll-like receptor (TLR) activation of B cells can initiate and potentially sustain normal B cell functions, we examined functional outcomes of TLR7 and TLR9 signaling in CVID B cells.

Methods—TLR7-mediated, TLR7/8-mediated, and TLR9-mediated cell proliferation, isotype switch, and immunoglobulin production by control and CVID B cells or isolated naive and memory B cell subsets were examined. We quantitated TNF- α , IL-6, and IL-12 production in response to TLR1-9 ligands and measured IFN- α production by TLR7-stimulated PBMCs and isolated plasmacytoid dendritic cells (pDCs). IFN- β mRNA expression by TLR3-stimulated fibroblasts was assessed. Results: Unlike CD27⁺ B cells of controls, TLR7-activated, TLR7/8-activated, or TLR9-activated CVID B cells or isolated CD27⁺ B cells did not proliferate, upregulate CD27, or shed surface IgD. TLR-stimulated CVID B cells failed to upregulate activation-induced cytosine deaminase mRNA or produce IgG and IgA. TLR7-stimulated PBMCs and pDCs produced little or no IFN- α . Reconstituting IFN- α in TLR7-stimulated CVID B-cell cultures facilitated proliferation, CD27 upregulation, and isotype switch. These TLR defects are restricted because CVID PBMCs stimulated with TLR ligands produced normal amounts of TNF- α , IL-6, and IL-12; TLR3-mediated expression of IFN- β by CVID fibroblasts was normal.

Conclusion—Defective TLR7 and TLR9 signaling in CVID B cells and pDCs, coupled with deficient IFN- α , impairs CVID B cell functions and prevents TLR-mediated augmentation of humoral immunity *in vivo*.

Keywords

Common variable immunodeficiency; Toll-like receptor; memory B cell; plasmacytoid dendritic cell; isotype switch; IFN- α

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Common variable immune deficiency (CVID) is a primary immune defect characterized by reduced serum IgG, IgA, and/or IgM and lack of protective antibody. The molecular mechanisms accounting for the failure of B cell development have remained, for the most part, unknown, although autosomal recessive mutations in the inducible T-cell costimulator,¹ the receptor for B cell activating factor,² and CD19³ have elucidated the underlying genetic causes in a few cases. Mutations in transmembrane activator and calcium ligand interactor are found in about 8% of subjects, but the reasons for loss of B cell function in these subjects remain unclear, because the same mutations can also be found in healthy family members.^{4–6} Although most subjects with CVID have normal numbers of peripheral B cells, the majority have very reduced numbers of circulating CD27⁺IgD[−] (isotype-switched) memory B cells, a feature relating to diminished antibody production⁷ and possibly a greater likelihood of more severe clinical outcomes.^{8–10}

Memory B cells are normally generated in germinal centers in response to T-dependent or T-independent antigens, but similar to other antigen presenting cells, B cells express various Toll-like receptors (TLRs),^{11,12} conserved membrane proteins that facilitate an alternative means of B cell activation.¹³ One of the most extensively studied TLR-driven systems is TLR9 signaling in B cells, the natural ligand being unmethylated CpG motifs in DNA oligodeoxynucleotides (CpG-ODN).¹⁴ TLR9 triggering activates B cells, upregulates costimulatory molecules, causes secretion of IL-6 and IL-10, and promotes B cell survival. TLR9 activation, with or without ligation of the B cell receptor (BCR), also mediates T-independent isotype switch by increasing transcription of activation-induced cytidine deaminase (AID); initiation of germline *Cγ1*, *Cγ2*, and *Cγ3* gene transcription; DNA recombination; and differentiation to antibody-secreting plasma cells.^{13,15–22} BCR activation enhances TLR9 expression in memory B cells, facilitating an augmented secondary antibody response on antigen rechallenge. TLR9 activation also upregulates its own expression, reinforcing the possibility of antibody production independent of the stimulating antigen.^{11,12,23} Signaling via the related, endosomal RNA-binding TLR7 also activates B cells, although with somewhat different requirements.^{24–26} TLR7 binds single-stranded RNA and synthetic agonists such as loxoribine,²⁷ a guanosine analog, and CL097, an imidazoquinoline derivative. CL097 also engages human TLR8, which uses a signaling pathway structurally similar to TLR7.²⁷

Because TLR activation appears to be an integral mechanism for activation and subsequent survival of memory B cells,²⁸ our previous investigation in CVID for TLR9 defects revealed that CVID B cells were not activated by CpG-ODN, even when costimulated by the BCR.²⁹ Here, we show that TLR7-stimulated, TLR7/8-stimulated, and TLR9-stimulated CD27⁺ as well as CD27[−] CVID B cells lack TLR-driven proliferation, AID expression, isotype switch, and immunoglobulin production. TLR7-triggered, TLR7/8-triggered, or TLR9-triggered CVID plasmacytoid dendritic cells (pDCs) also produce very little, if any, IFN- α . However, when this cytokine is added back into cell cultures, proliferation and generation of isotype-switched CD27⁺ B cells can be restored for some subjects. These studies demonstrate that the loss of TLR7 and TLR9 function is likely to be a critical defect in CVID.

METHODS

Patients and controls

Peripheral blood samples were obtained from subjects with CVID, age 18 to 87 years (see this article's Table E1 in the Online Repository at www.jacionline.org), by using an institutional review board–approved protocol and written informed consent. Diagnostic criteria for CVID included reduced serum IgG, IgA, and/or IgM 2 or more CIs below the normal ranges for age and verified antibody deficiency.³⁰ All subjects were healthy and receiving ongoing replacement immunoglobulin intravenously at intervals of 3 to 4 weeks.

Blood studies were performed before these treatments. Healthy adult volunteers and normal blood bank donors served as controls.

Cell isolation and culture

PBMCs were freshly isolated from heparinized peripheral blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). CD19⁺ B cells were positively selected by using immunomagnetic bead isolation (Miltenyi Biotec, Auburn, Calif). If sufficient B cells were obtained, CD19⁺CD27⁺ memory and CD19⁺CD27⁻ naive B cells were isolated by using positive microbead selection (Miltenyi Biotec), shown previously to not activate CD27⁺ memory B cells.³¹ The purity of isolated populations ranged from 60% to 78% for CD27⁺ B cells and 65% to 88% for CD27⁻ B cells by flow cytometry. Blood dendritic cell antigen (BDCA)-4/neuropilin-1–conjugated magnetic beads (Miltenyi Biotec) were used to isolate the pDCs as previously described.²⁹ All cell stimulations were performed at 37°C in RPMI 1640 medium (GIBCO, Carlsbad, Calif) with L-glutamine and 10% heat-inactivated FCS. Fibroblast cell lines from CVID and control subjects were established from 2-mm skin biopsies taken from the lower back and maintained in Dulbecco modified Eagle medium (GIBCO) with 20% FBS and 1% penicillin/streptomycin.

B cell proliferation, differentiation, and isotype switch

Isolated CD19⁺CD27⁺ and CD19⁺CD27⁻ B cell fractions were suspended in prewarmed PBS containing 0.5% BSA and labeled with 5-μmol/L carboxyfluorescein succinimidyl ester (Invitrogen, Carlsbad, Calif) for 5 minutes at room temperature, washed with 0.5% BSA/PBS, and cultured in complete medium with the optimum amount of loxoribine (500 μmol/L)^{27,32} (InvivoGen, San Diego, Calif) in the presence or absence of an optimum concentration of IFN-α (1000 U/mL; Schering, Kenilworth, NJ). After 6 days, flow-cytometric analysis was performed by gating on viable CD19⁺ cells or CD19⁺CD27⁺ and CD19⁺CD27⁻ populations. Division slicing³³ and surface expression of CD27 and IgD were analyzed by using FlowJo software (Ashland, Ore) to enumerate CD27⁻ naive, CD27⁺IgD⁺ memory, and CD27⁺IgD⁻ isotype-switched memory B cells⁸ in the proliferating cell populations.

TLR-induced IgG and IgA production

PBMCs (5×10^6 cells/mL) were stimulated in complete medium as above with 500 μmol/L loxoribine, 0.25 μg/mL CL097, or 0.6 μg/mL ODN2006 for 13 days. IgG and IgA content in the cell supernatants was determined by ELISA with a detection limit of 7.8 ng/mL (Bethyl Laboratories, Montgomery, Tex).

TLR-induced expression of AID mRNA

To examine TLR-mediated AID mRNA expression in CVID, 1×10^6 B cells were cultured with 500 μmol/L loxoribine, 0.25 μg/mL CL097, or 0.6 μg/mL ODN2006 for 24, 48, or 72 hours in the presence or absence of 1000 U/mL IFN-α. After stimulation, mRNA was isolated (RNeasy Mini Kit; Qiagen, Valencia, Calif) and reverse-transcribed (SuperScript III First-Strand cDNA synthesis kit, Invitrogen). Quantitative real-time PCR was performed by using the LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics, Indianapolis, Ind). RT-PCR products were also examined in isolated CD27⁺ and CD27⁻ B cells by agarose gel electrophoresis. The following primers were used: AID (forward, 5'-TGCTCTTCCTCGGCTACATCTC-3'; reverse, 5'-AACCTCATACAGGGGCAAAGG-3') and β-actin (forward, 5'-CTGAACCCCAAGGCCAACAG-3'; reverse, 5'-CCAGAGAAGAGGAGGATGCG-3').³⁴

TLR-induced cytokine production

To examine global TLR functions, control and CVID PBMCs at $2 \times 10^5/\text{mL}$ were stimulated with the following individual TLR ligands: 1, 5, and 10 $\mu\text{g/mL}$ Pam3CSK4 (TLR1); 0.4, 1.0, and 2.0 $\mu\text{g/mL}$ zymosan from *Saccharomyces cerevisiae* (TLR2 and TLR6); 0.2 to 20 $\mu\text{g/mL}$ polyriboinosinic polyribocytidylic acid (poly(I:C); TLR3); 0.02, 0.2, and 2 $\mu\text{g/mL}$ LPS from *Escherichia coli* (TLR4); 0.2, 0.5, and 10 $\mu\text{g/mL}$ flagellin from *Salmonella enterica* (TLR5); 10, 300, and 500 $\mu\text{mol/L}$ 7-allyl-7,8-dihydro-8-oxo-guano-sine (loxoribine; TLR7); 0.25 and 0.5 $\mu\text{g/mL}$ CL097 (TLR7/8); or 0.3, 2, 3, and 30 $\mu\text{g/mL}$ ODN2006 (TLR9; all from InvivoGen).³⁵ PBMCs stimulated with 20 ng/mL phorbol 12-myristate 13-acetate (Sigma, St Louis, Mo) and 1 $\mu\text{g/mL}$ ionomycin (Sigma) served as positive controls; cells in medium alone served as negative controls. After 24 hours, TNF- α , IL-12, and IL-6 levels were analyzed in supernatants by ELISA (BD Biosciences, San Diego, Calif). The detection limits were 7.8 pg/mL for TNF- α , 7.8 pg/mL for IL-12, and 4.7 pg/mL for IL-6.

IFN- α production by TLR7-stimulated isolated pDCs after 48 hours was quantified in harvested supernatants by ELISA (Bender Medsystems, Burlingame, Calif). To evaluate TLR3-mediated production of IFN- β , another type I IFN,³⁶ confluent CVID, and normal fibroblasts, which bear TLR3 but not TLR7, TLR8, or TLR9, were stimulated with poly(I:C) for 24 hours. Afterward, mRNA was isolated, cDNA was synthesized, and RT-PCR was performed as described by using these primers: IFN- β (5'-TTCCTGCTGTGCTTCTCCAC-3', reverse, 5'-GATTCCTACTACCAGTCCCAGAGTC-3').³⁷

Statistical analysis

Statistical analyses were performed by using GraphPad Prism v.4.03 (GraphPad Software Inc, San Diego, Calif). Data were expressed as mean values and SDs, ranges, and as needed, 10th percentiles and 25th (inter-quartile) percentiles. The Mann-Whitney test was used to compare cytokine and immunoglobulin production between CVID and control subjects. A P value $<.05$ was considered statistically significant.

RESULTS

TLR7-induced cell proliferation and differentiation is defective in CVID

As expected, TLR7-stimulated, and, to some extent, non-stimulated CD27⁺ control B cells exhibited greater proliferative responses than the CD27⁻ control B cells (Fig 1, *top panel*).^{26,32} CD27 expression was diminished on undivided CD27⁺ control and CVID B cells after 6 days; however, subsequent generations of divided TLR7-activated CD27⁺ control B cells gained high levels of CD27 and downregulated IgD, indicating isotype class switch (Fig 1, *middle and bottom panels*). In contrast, TLR7-activated CD27⁺ CVID B cells underwent fewer cell divisions, retained IgD, and failed to acquire CD27. For CVID B cells, the proliferative and surface expression pattern of the TLR7-stimulated and nonstimulated CD27⁺ and CD27⁻ populations were similar.

Decreased TLR-induced AID mRNA expression

The TLR7, TLR7/8, and TLR9 agonists did not upregulate AID mRNA expression in isolated B cells from 9 of 13 subjects with CVID compared with control B cells at the optimal time point of 72 hours (ODN2006; $P = .0031$; loxoribine, $P = .0274$; CL097, $P = .0014$; Fig 2, A). Because ODN2006 induced the highest expression of AID mRNA, we compared TLR9-mediated AID mRNA expression in isolated CD27⁺ and CD27⁻ control and CVID B cell populations. CD27⁺ control B cells had readily detectable AID mRNA after TLR9 stimulation, whereas CD27⁺ CVID B cells did not (Fig 2, B).

Defective TLR7-induced, TLR7/8-induced, and TLR9-induced IgG and IgA production

Although TLR7-mediated, TLR7/8-mediated, and TLR9-mediated activation causes normal B cells to produce immunoglobulin,^{26,32,38} CVID B cells produced very little IgG or IgA after stimulation with ODN2006 (IgG, $P = .007$; IgA, $P = .0016$), loxoribine (IgG, $P < .0001$; IgA, $P < .0001$), or CL097 (IgG, $P = .0166$; IgA, $P = .0014$; Fig 3).

Impaired TLR7-mediated production of type I IFN

Normal pDCs stimulated with the appropriate TLR agonists are the primary source of type I IFN in PBMC cultures.^{39,40} We found that CVID PBMCs produced significantly lower amounts of IFN- α over a range of loxoribine concentrations compared with controls (10 $\mu\text{mol/L}$, $P = .4520$; 300 $\mu\text{mol/L}$, $P = .0002$; 500 $\mu\text{mol/L}$, $P = .0012$; Fig 4, A). Isolated CVID pDCs were especially deficient in IFN- α production on TLR7 activation (100 $\mu\text{mol/L}$, $P = .0048$; 500 $\mu\text{mol/L}$, $P < .0001$; 1000 $\mu\text{mol/L}$, $P < .0001$; Fig 4, B). However, TLR3-activated CVID and control fibroblasts similarly upregulated IFN- β mRNA expression (Fig 5). Moreover, TLR3-triggered IFN- α production by CVID PBMCs was similar to controls when cultured 48 hours with poly(I:C) (0.5 $\mu\text{g/mL}$, $P = .7491$; 2 $\mu\text{g/mL}$, $P = .2707$; 20 $\mu\text{g/mL}$, $P = .8312$; data not shown). These results suggest that the TLR3 signaling pathway, another TLR-mediated pathway of type I IFN production, is likely to be intact in CVID.

IFN- α augments TLR7-induced class switch

Because minimal amounts of IFN- α had been detected in TLR7-stimulated and TLR9-stimulated²⁹ CVID PBMC and pDC cultures, we asked whether cell maturation and isotype switch in CVID B cells might be impaired because of a deficit of this cytokine. The addition of IFN- α to TLR7-activated CD27⁻ control B cells enhanced cell division (*top panel*), increased CD27 expression (*middle panel*), and downregulated IgD expression (*bottom panel*) to nearly the same extent as TLR7-stimulated CD27⁺ control B cells (Fig 6, A). The presence of IFN- α also markedly augmented B cell proliferation, gain of CD27, and loss of IgD (isotype switch) in CD27⁺ CVID B cells in a maturational pattern similar to CD27⁺ control B cells. For the isolated CD27⁻ CVID B cells, IFN- α enhanced cellular proliferation and loss of IgD, but we observed less upregulation of CD27 compared with the CD27⁻ control B cells. Whether the CVID B cells were cultured with loxoribine, IFN- α , or both, the percentage of isotype-switched B cells in these cultures was increased in 4 of 9 subjects with CVID into a range that overlapped with some of the controls (Fig 6, B).

Normal TLR-mediated production of inflammatory cytokines, TNF- α , IL-12, and IL-6

Common variable immunodeficiency PBMCs stimulated with the agonists for TLR1, TLR2, TLR3, TLR4, TLR5, TLR7, or TLR9 produced statistically similar quantities of TNF- α (see this article's Table E2 in the Online Repository at www.jacionline.org), IL-6 (see this article's Table E3 in the Online Repository at www.jacionline.org), and IL-12 compared with control PBMCs (data not shown), suggesting that general TLR activation is retained.

DISCUSSION

A fundamental defect in CVID is a block in B cell maturation, inhibiting immunoglobulin production and the development of plasma cells. Recent efforts have focused on elucidating the molecular defects that prevent normal heavy-chain isotype class-switching and somatic hypermutation in CVID. With the exceptions of autosomal recessive defects in a minority of subjects^{1,3} and the association with selected mutations in transmembrane activator and calcium ligand interactor,⁴⁻⁶ the pathogenesis of the CVID syndrome in most cases remains unknown. TLR activation leads to terminal B cell differentiation and immunoglobulin production⁴¹⁻⁴³ even in the absence of antigen exposure,^{44,45} but we had found that CpG-

ODN failed to activate TLR9 signaling in CVID B cells or promote IL-10 and IL-6 secretion. In the current studies, we find that functions of the related endosomal receptor, TLR7, on B cells is even more impaired, leading to poor B cell proliferation, lack of maturation, isotype switch, and IgG and IgA production. Naive B cells express lower levels of TLRs, whereas memory B cells constitutively express TLR7 and 9 at higher levels.^{11,20,38,43} The somewhat smaller proportion of circulating memory CD27⁺ B cells in CVID could account for the reduced TLR responses in CVID; however, we show that even isolated CD27⁺ CVID B cells have severely defective responses compared with CD27⁺ B cells of control subjects. These data suggest that intrinsic defects of TLR activation either accompany or lead to ineffective generation of antibody-producing isotype-switched B cells in CVID, whereas other TLR-mediated responses remain intact. Furthermore, these defects are not restricted to B cells because CVID pDCs produce little IFN- α on TLR7 and TLR9²⁹ triggering.

Because TLR7 and TLR9 signaling pathways are markedly defective in CVID, we suggest that 1 or more effector molecules common to these related activation pathways might be abnormal. TLR signaling requires an array of adapter molecules including myeloid differentiation factor (MyD88), myeloid differentiation factor adapter-like protein, Toll and IL-1 receptor domain–continuing adapter-inducing IFN- β (TRIF), and TRIF adaptor molecule. The potential importance of TLR function in B cell responses has been suggested by the reduction or elimination of T-dependent antibody responses in MyD88 knockout mice.⁴⁶ Functional differences and variable expression patterns of TLRs on different cell types have been important in dissecting mutations of the genes encoding components of the human TLR pathways, including nuclear factor- κ B (NF- κ B) essential modulator (NEMO/IKK γ), IL-1 receptor-associated kinase 4 (IRAK-4), inhibitor of NF- κ B, alpha (IkB α), MyD88, UNC93B, and TLR3.^{47–52} Mutations in these genes result in discrete immune defects that predispose the patient to selected bacterial, viral, and/or myco-bacterial infections. Because CVID PBMCs had normal TNF- α , IL-12, and IL-6 production, the intracellular signaling proteins such as MyD88 or NEMO/IKK γ that mediate NF- κ B activation by multiple TLRs were more likely to be preserved. However, even here, exceptions occur: although NEMO mutation classically manifests with defects in production of TNF- α and IL-6,⁴⁸ Salt et al⁵² identified 1 case with spared inflammatory cytokine production. The defects in CVID are somewhat similar to those found in subjects with mutations in the endoplasmic membrane protein UNC93B; the PBMCs of these patients also have impaired production of IFN- α on TLR7, TLR8, and TLR9 signaling but normal production of both TNF- α and IL-6.⁴⁹ However, in contrast with subjects with mutations in UNC93B, severe herpes simplex or other systemic viral infections are not common in CVID, possibly because the TLR3 pathway in CVID appears to be spared. We show that TLR3 activation of CVID PBMCs and fibroblasts leads to normal production of IFN- α and IFN- β , potentially permitting adequate viral protection. Although TLR7, TLR8, and TLR9 signal via the common adaptor protein MyD88, TLR3 signals via an MyD88-independent pathway involving TRIF and interferon response factor (IRF3).¹⁴ These differences may aid in further dissecting the TLR defects in CVID. Considering the endocytic location of these receptors,¹⁴ it is also possible that the intracellular trafficking, delivery, and/or processing of TLR ligands may be impaired in CVID.

A critical link between pDC and normal B cell function is the production of IFN- α . Similar to B cells, pDCs express high levels of TLR7 and TLR9; viral or ligand stimulation of these receptors normally results in the secretion of large amounts of type 1 IFNs. Although IFN- α is generally viewed as an antiviral or antiproliferative cytokine,⁵³ IFN- α enhances B cell sensitivity to TLR7/8-induced activation, primes B cells by enhancing responsiveness to BCR cross-linking, and further augments TLR-mediated isotype switch and immunoglobulin secretion.^{32,54–56} Because we found that CVID PBMCs and pDCs produce

little or no IFN- α when exposed to these TLR ligands, we tested whether adding IFN- α to TLR7-stimulated cell cultures might restore some facets of B cell function. In fact, IFN- α enhanced TLR7-induced CVID naive and, in particular, memory B cell proliferation in most subjects and increased the percentage of isotype-switched memory B cells in these cultures. On the other hand, restoration of IFN- α alone is not likely to improve IgG and IgA production *in vitro* because normal B cells triggered by TLR7 require additional cytokines, such as IL-2 and IL-10, to produce immunoglobulin.²⁶ However, these data suggest that the loss of IFN- α is an important component of this immune deficiency phenotype.

We demonstrate here that the CVID phenotype includes a selected impairment of responses of TLR7 and TLR9, the predominant TLRs expressed in pDCs and B cells. These defects include loss of cell activation, proliferation, and cytokine production by B cells and pDCs; as a result, CVID B cells forfeit the reinforcement that environmental TLR ligands provide normal human B cells. Our data suggests that the defects in TLR signaling in CVID could play a substantial role in the pathogenesis of this immunodeficiency.

Key messages

- Functional defects in TLR7 and 9 signaling in CVID lead to impaired B cell proliferation and differentiation and subsequent loss of switched memory B cells.
- Common variable immunodeficiency plasmacytoid dendritic cells produce little IFN- α , but reconstitution of this cytokine in TLR7-stimulated B cell cultures can induce proliferation, CD27 expression, and, in some cases, isotype switch.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AID	Activation-induced cytosine deaminase
BCR	B cell receptor
CpG-ODN	CpG motifs in DNA oligodeoxynucleotides
CVID	Common variable immunodeficiency
MyD88	Myeloid differentiation factor
pDC	Plasmacytoid dendritic cell
Poly(I	C), Polyriboinosinic polyribocytidylic acid
TLR	Toll-like receptor
TRIF	Toll and IL-1 receptor domain–continuing adapter-inducing IFN- β

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FIG 1.

Impaired CVID CD27⁺ B cell proliferation and differentiation. Isolated CD27⁺ and CD27⁻ CVID or control B cells were cultured with (Loxoribine) or without (No Stim) 500 mmol/L loxoribine for 6 days. CFSE dye dilution and cell surface expression of CD19 (*top panel*), CD27 (*middle panel*), and IgD (*bottom panel*) were tracked with each cell division. These data are representative of 12 subjects with CVID and 7 control subjects.

FIG 2.

A, Deficient AID expression in TLR-stimulated CVID B cells. AID expression by real-time PCR in isolated total B cells from subjects with CVID ($n = 10-13$) and control subjects ($n = 11$) after 72 hours of stimulation with ODN2006, loxoribine, or CL097. AID mRNA copy number was expressed as copy number per microgram of RNA normalized to β -actin. **B**, AID expression in TLR9-stimulated CD27⁻ and CD27⁺ B cells. AID expression by RT-PCR in isolated CD27⁺ and CD27⁻ B cells from subjects with CVID ($n = 6$) and control subjects ($n = 5$) after 72 hours of stimulation with ODN2006. AID mRNA was analyzed by agarose gel electrophoresis in comparison with β -actin. One representative subject with CVID and a control subject are depicted.

FIG 3.

Impaired TLR-induced IgG and IgA production by CVID B cells. CVID or control PBMCs were stimulated with ODN2006 (11 controls, 24 CVID), control ODN2137 (data not shown), loxoribine (11 controls, 24 CVID), or CL097 (6 controls, 16 CVID). IgG and IgA production was assessed by ELISA after 13 days.

FIG 4.

A, Impaired production of IFN- α by TLR7-stimulated CVID PBMCs. CVID (n = 14–24) and control (n = 9–10) PBMCs were cultured with 10 $\mu\text{mol/L}$, 300 $\mu\text{mol/L}$, or 500 $\mu\text{mol/L}$ loxoribine for 24 hours. **B**, Impaired production of IFN- α by TLR7-stimulated CVID pDCs. Isolated CVID (n = 27–34) and control (n = 20) pDCs were stimulated with 10 $\mu\text{mol/L}$, 300 $\mu\text{mol/L}$, or 500 $\mu\text{mol/L}$ loxoribine for 48 hours.

FIG 5.

TLR3-mediated IFN- β mRNA production by CVID fibroblasts is spared. Fibroblasts from subjects with CVID ($n = 4$) and control subjects ($n = 2$) were either untreated (–) or stimulated (+) with poly(I:C) for 24 hours. IFN- β mRNA was analyzed by agarose gel electrophoresis in comparison with β -actin. Data from 2 subjects with CVID and a representative control are shown.

FIG 6.

A, Addition of IFN- α amplifies TLR7-stimulated proliferation and differentiation of CD27⁺ and CD27⁻ B cells. Isolated CD27⁺ and CD27⁻ CVID or control B cells were stimulated with 500 μ mol/L loxoribine in the presence or absence of 1000 U/mL IFN- α for 6 days. CFSE dye dilution and cell surface expression of CD19, CD27, and IgD were tracked with each cell division. These data are representative of 12 subjects with CVID and 7 control subjects. **B**, TLR7-mediated isotype switching can be augmented in the presence of IFN- α . Total CD19⁺ B cells were stimulated with 500 μ mol/L loxoribine in the presence or absence of IFN- α for 6 days. CD27 and IgD surface expression was examined by flow cytometry to

determine the percentage of isotype-switched CD27⁺IgD⁻ B cells (*lower right quadrant of the inset contour plot*).