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Transcriptome Analysis of Peripheral Blood in Chronic Inflammatory Demyelinating Polyradiculoneuropathy Patients Identifies TNFR1 and TLR Pathways in the IVIg Response

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Abstract: We have studied the response to intravenous immunoglobulins (IVIg) by a transcriptomic approach in 11 chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) patients (CIDP duration = 6 [0.83–6.5] years). RNA was extracted from cells in whole blood collected before and 3 weeks after IVIg treatment, and hybridized on Illumina chips. After RNA quality controls, gene expression was analyzed using statistical tests fitted for microarrays (R software, limma package), and a pathway analysis was performed using DAVID software. We identified 52 genes with expression that varied significantly after IVIg (fold change [FC] > 1.2, $P < 0.001$, false discovery rate [FDR] <0.05). Among these 52 genes, 7 were related to immunity, 3 were related to the tumor necrosis factor (TNF)-α receptor 1 (TNFR1) pathway (inhibitor of caspase-activated DNase (ICAD): FC = 1.8, $P = 1.7E-7$, FDR = 0.004; p21 protein-activated kinase 2 [PAK2]: FC = 1.66, $P = 2.6E-5$, FDR = 0.03; TNF-α-induced protein 8-like protein 1 [TNFAIP8L1]: $P = 1.00E-05$, FDR = 0.026), and 2 were related to Toll-like receptors (TLRs), especially TLRs 7 and 9, and were implicated in autoimmunity. These genes were UNC93B1 (FC = 1.6, $P = 2E-5$, FDR = 0.03), which transports TLRs 7 and 9 to the endolysosomes, and RNF216 (FC = 1.5, $P = 1E-05$, FDR = 0.03), which promotes TLR 9 degradation. Pathway analysis showed that the TNFR1 pathway was significantly lessened by IVIg (enrichment score = 24, Fischer exact test = 0.003). TNF-α gene expression was higher in responder patients than in nonresponders; however, it decreased after IVIg in responders ($P = 0.04$), but remained stable in nonresponders. Our data suggest the actions of IVIg on the TNFR1 pathway and an original mechanism involving innate immunity through TLRs in CIDP pathophysiology and the response to IVIg. We conclude that responder patients have stronger inflammatory activity that is lessened by IVIg.

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Abbreviations: CIDP = chronic inflammatory demyelinating neuropathy, CISP = chronic inflammatory sensitive neuropathy, DC = dendritic cell, FC = fold change, FDR = false discovery rate, IFN = interferon, IVIg = intravenous immunoglobulin, PBMC = peripheral blood mononucleated cells, RNA = ribonucleic acid, TLR = Toll-like receptor, TNFR = tumor necrosis factor receptor.

INTRODUCTION

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an immune-mediated disorder of peripheral nerves. It is a disabling condition, causing motor deficits and sensory disorders with various clinical presentations.[1] Its physiopathology remains largely unknown. The implication of cellular immunity is suspected,[2,3] as macrophages and T CD4+ lymphocytes have been found in nerve biopsies of CIDP patients. Moreover, it was recently shown that the T-cell receptor repertoire of cells infiltrating peripheral nerves have a strong monoclonal or oligoclonal restriction that corresponds to that of circulating T cells.[4] These data support the hypothesis of an antigen-driven T-cell attack against nerves, even if the target of this immune response remains unclear. Humoral immunity could also be implicated, as indirectly supported by the beneficial effects of plasmapheresis[5] and by the fact that conduction blocks were induced in rats by injecting purified immunoglobulin (Ig)G from CIDP patients.[3] Several antibodies directed against myelin components or nodal and paranodal proteins have been reported, but only in a small number of CIDP patients.[2,6]

Intravenous immunoglobulins (IVIg) are a first-line therapy for CIDP.[7] IVIg have several mechanisms of action that are still not completely understood.[8,9] It has been shown that IVIg modify autoimmunity by modulating several functions and cells, including immunoglobulin fold change (FC) fragment receptor expression,[10]

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monocyte and macrophage activation, differentiation and maturation of dendritic cells (DCs), regulatory T cells, autoreactive B lymphocytes,[11] auto and allo-reactive T cells,[9,12] complement-mediated damage,[8] and the levels of circulating autoantibodies.[9] Furthermore, the therapeutic effects of IVIg may result from different mechanisms depending on the pathophysiology of the autoimmune disease in which it is used.

In CIDP, it has been shown that IVIg up-regulate the expression of Fcgamma RIIb on naïve B cells,[13] reduce autoreactive T-cell responses against PMP-22 and P2-antigens,[12] and regulate Bcell activating factor expression.[14] However, the biological results of these observations and their relationship with the disease physiopathology remain poorly understood.

Here we use a transcriptomic approach to compare the gene expression profiles in the peripheral blood of CIDP patients before and after IVIg treatment to identify their mechanisms of action in this condition, to lead to a better understanding of CIDP pathophysiology, and to potentially determine the factors associated with the IVIg response in CIDP patients.

METHODS

Patient Selection and Inclusion Criteria

Patients were consecutively recruited at the Pitié-Salpêtrière Hospital (Paris, France) in a French National Reference Centre for Rare Neuromuscular Disorders. Samples were collected from all patients seen between June 2013 and 2014 who matched the inclusion criteria and agreed to take part in our study. The patients signed an informed consent before participating in any procedure. The project was approved by the local ethics committee (CPP Ile de France VI) on May 30, 2012. Inclusion criteria included the following: a definite or probable CIDP diagnosis using the 2010 European Federation of Neurological Societies (EFNS)/Peripheral Nerve Society (PNS) diagnostic criteria or an atypical CIDP diagnosis meeting the EFNS/PNS clinical criteria and at least 2 EFNS/PNS supportive criteria[7]; and indication of IVIg treatment.

Samples Collection

A peripheral blood sample was collected from each patient using a Paxgene tube at baseline (T1), that is, 1 hour before IVIg administration. For patients treated with IVIg before their inclusion in the study, the T1 sample was collected after a 6-week washout period. The treatment consisted of 2 g/kg of IVIg administered over a 3-day period. A second blood sample (T2) was then collected for each patient using a Paxgene tube. In these patients, the T2 sample was collected 3 weeks after T1. For patients in which IVIg treatment was just initiated (naïve patients), we administered a first course of IVIg (2 g/kg) 1 hour after T1, followed by a second course (2 g/kg) 4 weeks later. The T2 sample was collected 3 weeks after the second course of IVIg, ensuring better comparisons with patients already under long-term IVIg treatment. The samples were frozen within 24 hours and stored at -80°C until further use.

Patients were evaluated at the T1 and T2 time points using the Overall Neuropathy Limitations Scale (ONLS), the Medical Research Council (MRC) scale for muscle strength, and the inflammatory neuropathy cause and Treatment sensory sum score. A patient was considered a responder to IVIg if the ONLS score decreased by at least 1 point at the T2 time point compared with T1.

RNA Processing

RNA was extracted from PAXgene tubes using the PAXgene Blood RNA kit (Qiagen) followed by a cleaning with the RNeasy MinElute Cleanup kit (Qiagen), according to the manufacturer's instructions. RNA was quantified by 260/280 nm absorbance (nanodrop), and RNA quality was controlled by electrophoresis in an Agilent Bioanalyzer. RNA (250 ng) was amplified with the TotalPrep Illumina kit (Ambion, Life Technologies), and 1.5 μg cDNA was hybridized to Illumina Human HT-12 BeadChip arrays (Illumina). The results were read by an Illumina BeadArray scanner at the genomics facility of the Pitié-Salpêtrière Hospital (P3S). To minimize batch effects, a maximum of samples were processed at the same time, buffers from the different kit batches were pooled, and the order in which the samples were processed was randomized. The position of each sample on the chips was also randomized, and the paired samples of each patient were assigned to the same chip. To confirm the microarray experiments results, real-time RT-PCR was performed on 5 genes of interest (UNC93B1, RNF216, ICAD, PAK2, and tumor necrosis factor [TNF]-α) using the Prism 7500 HT sequence detection system (Applied Biosystems). quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed on only 8 patients because insufficient quantities of RNA were available for the other 3 patients. Primers were designed with the Oligo Explorer 1.0 software and verified for specificity with the NCBI Blast engine (www.ncbi.nlm.nih.gov/BLAST) using the "nearly exact short match" program. The PCR products were analyzed with the SDS 2.1 real-time detection system software. The $18-28$ Ct method was used for quantification. The relative levels of mRNA were standardized using RPL13 RNA as the nonvariant RNA species.

Statistical Analysis

Quality control was performed using the lumi R package (box plot of intensities, clustering), and Bead Studio software (p95/p05, p95/background, BeadStudio, Illumina). Reproducibility was verified by hybridizing duplicate membranes. The nonspecific background was suppressed; the data were converted to log2 and then normalized using the median intensity of the arrays. The threshold intensity was set at a minimum value of 10. The statistical analysis to determine the gene expression profile before and after treatment was performed with the limma package (R software) using a paired *t* test fitted for microarrays. The *P* values were adjusted by the Benjamini and Hochberg method,[15] correcting for multiple comparisons (false discovery rate [FDR]) with a significance threshold set at $P = 0.001$ and $FDR = 0.05$. We selected as genes of interest those with expression that varied (FC) by more than 1.2 times the median and were significantly deregulated by the treatment ($P < 0.001$ and $FDR < 0.05$). A positive FC indicated the gene expression was decreased after IVIg treatment, whereas a negative FC signified that the gene expression was increased after IVIg treatment. The principal biological processes deregulated in our samples were determined by a comparison between a list of deregulated genes and a list of reference genes using the DAVID Bioinformatics Resources 6.7 Functional Annotation Tool (NIAID/NIH).

Variation in gene expression between the responder and nonresponder patients was compared using a Welch *t* test (R). Values are expressed as the median ± interquartiles for the clinical characteristics patients and as the mean ± standard deviation for gene expression values.

RESULTS

Patients

Eighteen patients were included in this study. Blood samples (PAXgene) were collected before (T1) and 3 weeks

(T2) after IVIg treatment. Among the 36 samples that were processed, 7 samples were excluded from subsequent analysis because of poor RNA quality after extraction (4 samples), insufficient yield after RNA amplification (1 sample), or the hybridization median intensity was out of range (2 samples). Eleven patients were included in the final analysis, including 7 men and 4 women who had a median age at the time of inclusion of 64 years (interquartile range [IQR] 60–71.5). Among these patients, the median duration of CIDP was 6 years [IQR 0.83–6.5]. Seven patients had a previous IVIg treatment established more than 5 months before their inclusion in the study (median duration of IVIg treatment 11 months [IQR 6–72]), and 4 patients were treatment-naïve and began IVIg treatment at the time of inclusion.

Three patients had the common form of CIDP, 4 patients had a Lewis–Sumner syndrome, 1 patient had a pure clinical motor form, and 3 patients had a pure clinical sensory form. All of the patients met the EFNS/PNS electrophysiological criteria, except 2 patients who presented a chronic immune sensory polyradiculopathy (CISP), which is an atypical variant of CIDP. One of these CISP patients met 2 supportive EFNS/PNS criteria, whereas the other met 3 of these criteria; both had a positive nerve biopsy. Seven patients were considered responders and 4 patients were nonresponders.

Gene Expression Profiling Before and After IVIg Treatment

We identified 52 genes with expression values that were significantly different between T1 and T2 ($P < 0.001$, FDR < 0.05; Table 1).

Most of the genes (96 %) were down-regulated at T2. This molecular signature clearly differentiated T1 and T2 samples (Figure 1). Significant genes were associated with immunity, transcription regulation, intracellular signaling, and other biological functions.

In particular, 7 genes were known to play a role in immunity. An unbiased pathway analysis using the Biocarta database revealed 2 significantly enriched pathways—the TNF-α receptor 1 (TNFR1) and Fas ligand pathways (enrichment score = 24, Fischer exact test = 0.003 for TNFR1; and enrichment score = 23.2, Fischer exact test = 0.003 for Fas ligand). Indeed, 3 out of the 7 genes were associated with the TNF-α signaling pathway, including the DNA fragmentation factor (ICAD), PAK2, and TNFAIP8L1. We then performed a subsequent analysis restricted to genes involved in immunity and inflammation (687 genes according to Gene Ontology), and we found 9 significantly deregulated genes (Table 2), including the gene encoding TNF-α itself.

The qRT-PCR analysis confirmed the decrease in ICAD ($P = 0.016$), PAK2 ($P = 0.008$), and TNF-α ($P = 0.039$) gene expression after IVIg (Figures 2 and 3).

Interestingly, 2 of the 7 genes related to immunity were involved in Toll-like receptor (TLR) (especially TLR 7 and 9 implicated in autoimmunity) activity. These genes included UNC93B1 (FC = 1.6, $P = 2E\text{-}05$, FDR = 0.03), which regulates TLR activity by transporting TLR 7 and TLR 9 from the endoplasmic reticulum to the endolysosomes,[16] and RNF216 (FC = 1.5, $P = 1E\text{-}05$, FDR = 0.03), which promotes TLR 4 and TLR 9 degradation.[17] Each of these genes was down-regulated. Using qRT-PCR, we confirmed the decreased gene expression after IVIg for UNC93B1 ($P = 0.016$), but not for RNF216 ($P = 0.195$) (Figures 2 and 3).

The other 2 deregulated genes implicated in autoimmunity were hematopoietic cell signal transducer, which plays a role in triggering cytotoxicity,[18] and CD68, the function of which is still controversial, but it could be a component of an antigen presenting system.[19]

TNF-α Expression Decreases After IVIg in Responder Patients

Because the TNF-α pathway seemed to be the major immunological pathway regulated by IVIg in CIDP, we compared the change in its expression levels in responder and nonresponder patients. As shown in Figure 4, TNF-α gene expression decreased significantly after IVIg in responders compared with that in nonresponders ($P = 0.04$).

DISCUSSION

The gene expression levels in CIDP have been compared using a transcriptomic approach to control patients in nerve[20] and skin biopsies.[21,22] Renaud et al[20] performed a transcriptome analysis in sural nerve biopsies, comparing 8 CIDP patients with 6 controls. Most of the 123 differentially expressed genes were involved in immunity and signal transduction, including tachykinin precursor 1 (TAK1), which may be involved in pain mediation; stearoyl-CoA-desaturase (SCD), which may be a marker for remyelination; and the allograft inflammatory factor 1 (AIF-1), a modulator of immune response during macrophage activation.

In 2010, Lee et al[22] studied the transcriptomic profile on skin punch biopsies form 11 CIDP patients and 15 controls. Five up-regulated genes of interest were highlighted, which were related to inflammatory, immune, or defence processes. These genes encoded AIF-1, lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), FYN-binding protein (FYB), myeloid/lymphoid or mixed-lineage leukaemia (trithorax homolog, Drosophila); translocated to, 3 (MLLT3) and purinergic receptor P2Y, G-protein coupled, 1 (P2RY1).

More recently, Puttini et al[21] performed transcriptional profiling on skin punch biopsies from 20 CIDP patients and 17 healthy controls. Twenty-six genes were differentially expressed and were mostly involved in immune and chemokine regulation, growth, and repair. That study also showed the up-regulation of 2 genes, kinase insert domain receptor (a type III receptor tyrosine kinase) (KDR) and discoidin domain receptor tyrosine kinase 2 (DDR2), could be a biomarker of CIDP, and also the up-regulation of LYVE-1.

Our study is the first to employ a transcriptomic approach in blood cells of CIDP patients comparing gene expression levels before and after IVIg, with each patient being its own control. This enabled us to formulate hypotheses on the mechanisms of action of IVIg in CIDP and on CIDP pathophysiology.

In this study, we found a molecular signature induced by IVIg treatment in the whole blood of CIDP patients. The statistical analysis of the differentially expressed genes after IVIg treatment compared with baseline led to the identification of 52 genes of interest that should be further explored.

TNF-α Expression and TNFR1 Pathways are Modified by IVIg in CIDP

The TNFR1 pathway was significantly down-regulated by IVIg in our cohort, and 3 of the 7 down-regulated genes related to immunity belonged to the TNF-α signaling pathway (PAK2, ICAD, and TNFAIP8L1). Moreover, we found in responder

TABLE 1. Genes Significantly Deregulated After IVIg and Their Functions

Symbol	Name	Function	Fold Change	*P*	FDR
Immunity					
ICAD	DNA fragmentation factor	TNFR1 pathway	1.805	1.72E-07	0.004
PAK2	P21 protein (Cdc42/Rac)-activated kinase 2	TNFR1 pathway	1.657	2.59E-05	0.038
TNFAIP8L1	Tumor Necrosis Factor, Alpha-Induced Protein 8-Like	Cellular secretion and oncogenesis	1.644	1.00E-05	0.026
UNC93B1	Unc-93 homolog B1	TLR Transport	1.626	1.81E-05	0.032
RNF216	Ring finger protein 216	TLR 4 and 9 degradation	1.458	9.79E-06	0.026
HCST	Hematopoietic cell signal transducer	Triggering of cytotoxicity	1.439	4.56E-05	0.044
CD68	CD68 molecule	Possible component of an antigen presenting system	1.385	7.23E-06	0.024
Transcription					
RAI1	Retinoic acid induced 1	Regulation of transcription	1.726	3.52E-05	0.04
SF3B4	Splicing factor 3b, subunit 4	Splicing	1.693	3.02E-05	0.039
TROVE2	TROVE domain family, member 2	RNA binding	1.551	3.33E-06	0.024
C14orf4	Interferon regulatory factor 2 binding protein-like	Regulation of transcription	1.538	3.72E-05	0.04
RBM42	RNA-binding motif protein 42	RNA binding	1.455	3.44E-05	0.04
KHSRP	KH-type splicing regulatory protein	RNA binding	1.409	4.66E-06	0.024
SCAND1	SCAN domain containing 1	Regulation of transcription	1.408	3.80E-05	0.04
SCAND1	SCAN domain containing 1	Regulation of transcription	1.384	5.10E-06	0.024
PIAS4	Protein inhibitor of activated STAT, 4	Regulation of transcription	1.296	1.63E-05	0.03
E4F1	Transcription factor E4F1	Regulation of transcription	1.275	5.12E-05	0.047
SF3A2	Splicing factor 3a, subunit 2	Splicing	1.245	1.53E-05	0.029
Cell signaling					
SGK3	Serum/glucocorticoid regulated kinase family, member 3	Serine/threonine proteine kinase	1.41	1.51E-05	0.029
DUSP23	Dual specificity phosphatase 23	Tyrosine/serine/threonine phosphatase	1.383	3.73E-05	0.04
EVI5L	Ecotropic viral integration site 5-like	GTPase activation	1.377	4.79E-06	0.024
SBF1	SET binding factor 1	Rseudophosphatase	1.341	3.09E-06	0.024
AURKAIP1	Aurora kinase A interacting protein 1	Kinase Aurora A regulation	1.278	9.47E-06	0.026
JMJD7	Jumonji domain containing 7	Metalloenzyme	−1.542	1.48E-05	0.029
Other					
SPAST	Spastin	Micotubule cleavage	2.527	1.31E-07	0.004
TNNI2	Troponin I type 2	Muscular contraction	1.63	7.68E-06	0.024
GAS6	Growth arrest-specific 6	Cellular proliferation	1.623	2.66E-05	0.038
TRIOBP	TRIO and F-actin binding protein	Cytoskeleton organization	1.573	1.11E-05	0.027
HNRNPUL2	Heterogeneous nuclear ribonucleoprotein U-like 2	Nucleic acid binding	1.564	5.85E-06	0.024
BCL7B	B-cell CLL/lymphoma 7B	Actin binding	1.559	4.52E-05	0.044
PI4KB	Phosphatidylinositol 4-kinase	Golgi organisation	1.533	3.25E-06	0.024
PCSK5	Proprotein convertase subtilisin/kexin type 5	Posttranslational protein maturation	1.523	2.54E-05	0.038
MSRB2	Methionine sulfoxide reductase B2	Protection against oxidative stress	1.463	3.11E-05	0.039
PAPD5	PAP associated domain containing 5	DNA polymerase	1.411	5.62E-06	0.024
CHMP6	Charged multivesicular body protein 6	Endosomal vesicle sorting	1.405	5.44E-05	0.049
Magmas	Presequence translocase-associated motor 16 homolog	Protein translocation	1.391	1.87E-05	0.032
CAPZB	Capping protein (actin filament) muscle Z-line, beta	Cytoskeleton organisation	1.311	1.18E-05	0.028
PNPLA6	Patatin-like phospholipase domain containing 6	Neurite growth	1.294	3.35E-05	0.04

Symbol	Name	Function	Fold Change	P	FDR
MGAT1	Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	Development	1.293	2.86E-05	0.039
LFNG	LFNG O-fucosylpeptide 3-beta-N-acetyl glucosaminyl transferase	Development	1.263	3.37E-05	0.04
VKORC1	Vitamin K epoxide reductase complex subunit 1	Vitamin K activation	1.254	1.29E-05	0.028
TMED4	Transmembrane Emp24 protein transport domain containing 4	Vesicular protein trafficking	1.251	4.37E-05	0.044
CCM2	Cerebral cavernous malformation 2	Angiogenesis	1.248	6.66E-06	0.024
GSTP1	Glutathione S-transferase P	Xénobiotic metabolism	1.233	3.14E-05	0.039
Unknown					
C14orf102	Chromosome 14 open reading frame 102	Unknown	2.082	2.95E-05	0.039
LOC401115		Unknown	1.793	4.31E-06	0.024
LOC643384		Unknown	1.555	2.29E-05	0.036
ASMTL	Acetylserotonin O-methyltransferase-like	Unknown	1.451	4.64E-05	0.044
ESRRAP2	Estrogen-related receptor alpha pseudogene 2	Unknown	1.433	1.22E-05	0.028
TMEM189	Transmembrane protein 189	Unknown	1.375	2.03E-05	0.033
LOC374395		Unknown	1.208	6.74E-06	0.024
C14orf45	Chromosome 14 open reading frame 45	Unknown	−1.933	4.50E-05	0.044

FDR = false discovery rate, HCST = hematopoietic cell signal transduce, ICAD = inhibitor of caspase-activated DNase, IVIg = intravenous immunoglobulin.

patients that the TNF-α gene expression level was higher before IVIg treatment and decreased significantly after treatment.

Tumor necrosis factor-α is a cytokine produced by macrophages and activated T lymphocytes. It has a pro-inflammatory effect mediated by all major mediators of inflammation, including chemokines, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), and NF-κB (nuclear factor of kappa light polypeptide gene enhancer in B-cells)-regulated proteins, such as interleukin (IL)-6, IL-8, and IL-18. TNF-α activation of TNFR1 has a pro-inflammatory effect (induction of NF-κB), and induces cell apoptosis (recruitment of TRADD (tumor necrosis factor receptor 1 (TNFR1)-associated death domain protein), Fas associated via death domain, and then activation of caspases 8 and 3).[23]

The implication of TNF-α in CIDP has been suggested. Serum levels of TNF-α are increased in the active phase of clinically severe CIDP patients, but decreased after immunotherapy with IVIg or plasmapheresis.[24]

Previous findings showed that IVIg treatment causes a decrease in TNF-α production in lipopolysaccharide-stimulated peripheral blood mononuclear cells (PBMCs).[25] Yang et al[26] have shown that the expression of TNFR1 in B lymphocytes of CIDP patients decreases after IVIg treatment. Paradoxically, several reports have been published in which CIDP cases occurred after anti-TNF-α treatment.[27–29]

We showed that in responder patients, the TNF-α gene expression level was higher before IVIg treatment and decreased significantly after treatment. This suggests that patients who respond to IVIg are those who have stronger TNF-α expression, which is decreased by the treatment.

Future studies are necessary to determine if the TNF-α expression level is a marker of the IVIg response in CIDP patients. Conversely, a low TNF-α expression could be an indicator for IVIg discontinuation.

Potential IVIg-mediated TLR Modulation Through UNC93B1 and RNF216 Genes

In addition to the TNFR1 pathway modulation, we found that 2 genes involved in the regulation of TLR activity, UNC93B1 and RNF216, which are especially involved in TLR 7 and TLR 9 regulation, were down-regulated by IVIg.

The TLRs play a key role in the innate immune system. Activation of these receptors induces production of inflammatory cytokines, and also type I interferon (IFN). Loss of negative regulation and recognition of self molecules by TLRs have been shown to play a role in the pathogenesis of inflammatory and autoimmune diseases.[30] Our data suggest that IVIg regulates TLR activity in CIDP.

In particular, TLR 7 and TLR 9 are implicated in autoimmunity. Fukui et al[16] suggested that TLR 9 has a protective effect in autoimmune prone MRL/lpr mice and that its effect is mediated by competition with TLR 7 for interaction with UNC93B1. TLR 9 also has a pathogenic action of its own: it was shown to induce Th1 autoimmunity in a mouse model of antibody-associated vasculitis (AAV),[31] and was found to be crucial in the formation and maintenance of inflammatory lesions in an experimental allergic encephalomyelitis (EAE) mouse model.[32]

Toll-like receptor 7 has been shown to promote production of autoreactive antibodies in systemic lupus erythematosus (SLE) models, whereas TLR 9 has an opposing protective role,[33] controlling the TLR 7 response on B cells.[34]

In CIDP, however, the role of TLRs has not been reported, although it has been described that TLRs might be involved in Guillain–Barre syndrome, the acute form of CIDP, and that

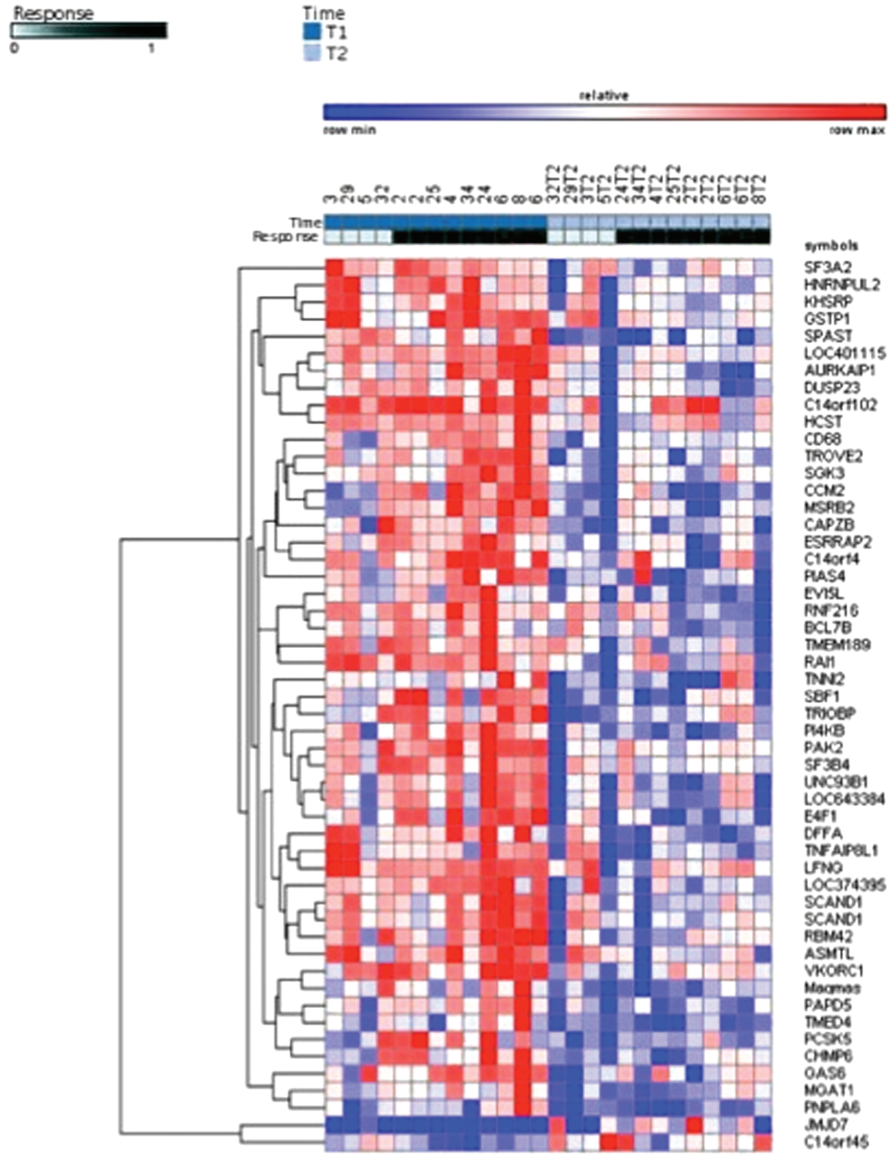

FIGURE 1. Molecular signature induced by IVIg. Heatmap featuring the expression of the 52 significantly deregulated genes identified in our primary analysis (FDR < 0.05). Genes represented in red are highly expressed (most genes before treatment), whereas genes represented in blue are weakly expressed (most genes after treatment). Responder patients are defined by a black square, nonresponders by a white square. FDR = false discovery rate, IVIg = intravenous immunoglobulin.

inactivating TLR 9 induces clinical improvement in experimental autoimmune neuritis.[35–37]

It is established that UNC93B1 controls differential trafficking of endosomal TLRs,[16,38] which may explain its role in autoimmunity. UNC93B1 actively and continuously regulates excessive TLR7 activation of immune cells by employing TLR 9 to counteract TLR 7.[16] The role of UNC93B1 in CIDP has not been reported in the literature, although some data exist in SLE. It has been shown that that UNC93B1 mRNA expression levels in PBMCs and B cells isolated from active SLE patients were significantly higher than those in healthy controls and inactive in SLE patients.[39]

RNF216 promotes TLR 4 and TLR 9 degradation,[17] and therefore modulates the balance between TLRs.

These results suggest that IVIg regulates TLR activity in CIDP. These findings are consistent with previous data reporting an action of IVIg on TLRs, leading to a decrease in pro-inflammatory cytokines. It has been demonstrated that IVIg decreases the production of pro-inflammatory cytokines, especially TNF-α and IL-6, by modulating the TLR 4 signaling pathway.[25] More recent data indicate that IVIg, more specifically their F (ab')2 fragment, decreases IFN-α production induced by TLR 7 and TLR 9 agonists.[40]

Study Limitations

The results presented in this article have several limitations, inherent to transcriptome analysis and the experimental

TABLE 2. Genes Belonging to Immunity and Inflammation Deregulated After IVIg

Symbol	FC	*P*	FDR
NLRP12	1.67	0.00024	0.02
CAMP	1.65	0.00054	0.03
GNA15	1.49	0.00021	0.02
NFKB2	1.42	0.00020	0.02
TNF-α	1.39	0.00013	0.02
NFKBIA	1.37	0.00032	0.03
GNAI2	1.36	0.00012	0.02
RRAS	1.35	0.00037	0.03
COL20A1	−1.45	0.00011	0.02

FC = fold change, FDR = false discovery rate, IVIg = intravenous immunoglobulin.

design. Performing the analysis on whole blood using PaxGene technology had the advantage of avoiding RNA degradation, but it does not allow us to determine the cellular subtype in which the gene expression modification occurs. Furthermore, the small number of patients included in this pilot study does not allow final conclusions on the present biological results and will need further validation in independent cohorts. A case-control study is currently under way to confirm our results.

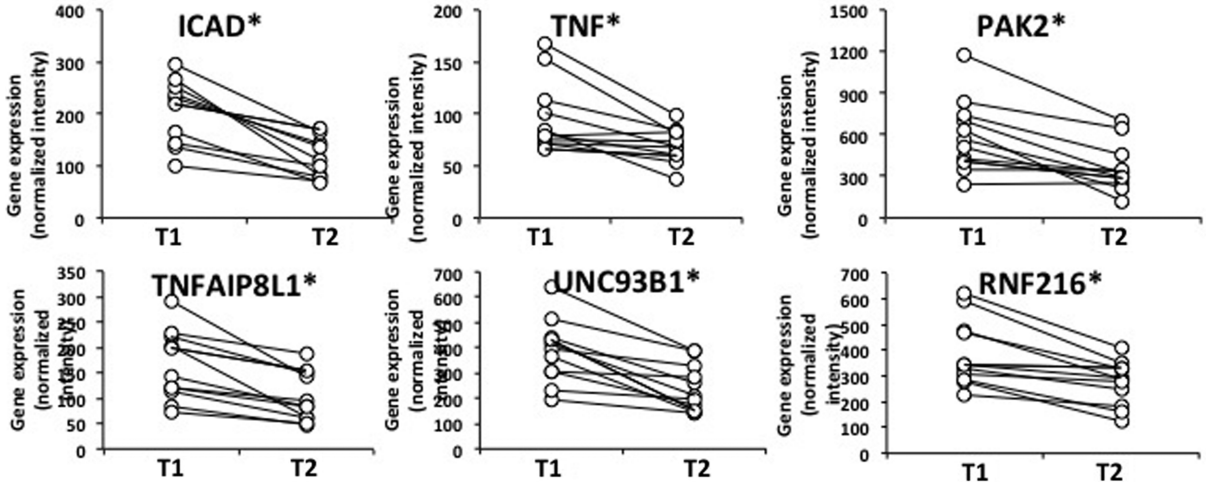

FIGURE 2. Expression of selected genes identified by a transcriptomic approach. Gene expression (normalized intensity) before (T1) and after (T2) treatment by IVIg for our genes of interest. IVIg = intravenous immunoglobulin.

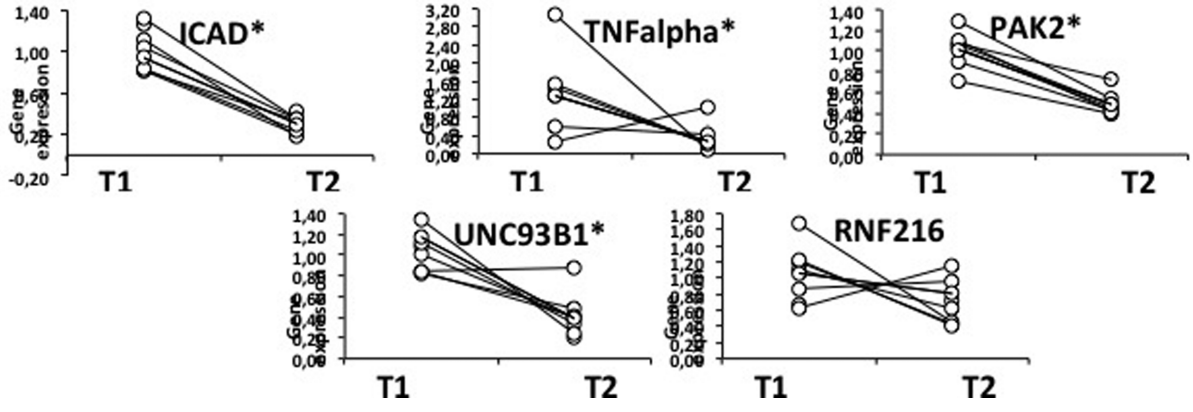

FIGURE 3. Expression of selected genes measured by qRT-PCR. Expression level of our genes of interest before (T1) and after (T2) treatment by IVIg. IVIg = intravenous immunoglobulin.

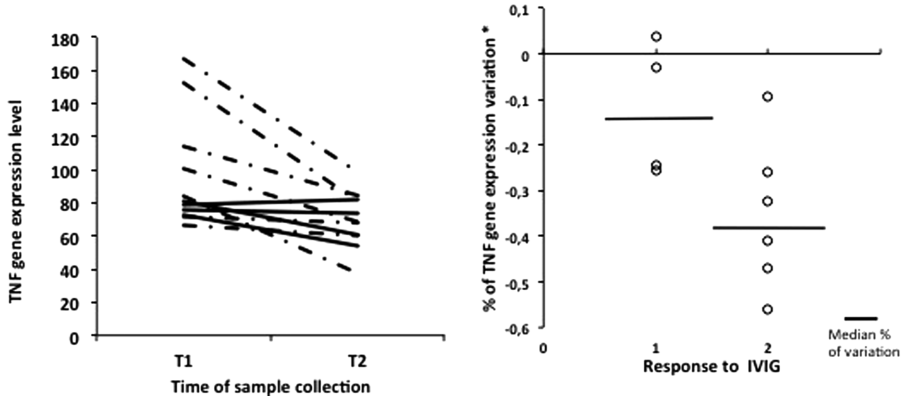

FIGURE 4. TNF-α gene expression and response to IVIg in CIDP patients. TNF-α gene expression levels before (T1) and after (T2) treatment measured by microarrays in whole blood (left panel). Continuous lines represent nonresponder patients and dotted lines represent responder patients. Change in TNF-α gene expression in responders and nonresponders expressed as the percentage of its expression before and after treatment by IVIg (right panel). $P = 0.04$, Welch t test. CIDP = chronic inflammatory demyelinating polyradiculoneuropathy, IVIg = intravenous immunoglobulin, TNF-α = tumor necrosis factor-α.

CONCLUSIONS

In conclusion, our study provides additional information on the role of IVIg in the TNFR1 pathway, and it has shown an original mechanism in CIDP pathophysiology and response to IVIg, implicating TLRs. Furthermore, we have established that TNF-α expression decreases after IVIg in responder patients. Although the findings should be confirmed by different methods in independent cohorts, these data provide promising insights into the physiopathology and response to IVIg in CIDP.

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