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Original Article

Tocilizumab attenuates inflammation in ALS patients through inhibition of IL6 receptor signaling

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Abstract: Patients with amyotrophic lateral sclerosis (ALS) have evidence of chronic inflammation demonstrated by infiltration of the gray matter by inflammatory macrophages, IL17A-positive T cells, and mast cells. Increased serum levels of IL6 and IL17A have been detected in sporadic ALS (sALS) patients when compared to healthy controls. Herein we investigate, in peripheral blood mononuclear cells (PBMCs), the baseline transcription of genes associated with inflammation in sALS and control subjects and the impact of the IL6 receptor (IL6R) antibody (tocilizumab) on the transcription and/or secretion of inflammation factors (e.g. cytokines) stimulated by the apo-G37R superoxide dismutase (SOD1) mutant. At baseline, PBMCs of four sALS patients (Group 1) showed significantly increased expression of TLR2 and CD14; ALOX5, PTGS2 and MMP1; IL1α, IL1β, IL6, IL36G, IL8 and TNF; CCL3, CCL20, CXCL2, CXCL3 and CXCL5. In four other sALS patients (Group 2), most of the genes just mentioned were expressed at near control levels and a significant decrease in the expression of PPARG, PPARA, RARG, HDAC4 and KAT2B; IL6R, IL6ST and ADAM17; TNFRSF11A; MGAT2 and MGAT3; PLCG1; CXCL3 were detected. Apo-G37R SOD1 up regulated the transcription of cytokines (e.g. IL1α/β, IL6, IL8, IL36G), chemokines (e.g. CCL20; CXCL3, CXCL5), and enzymes (e.g. PTGS2 and MMP1). In vitro, tocilizumab down regulated the transcription of many inflammatory cytokines, chemokines, enzymes, and receptors, which were up regulated by pathogenic forms of SOD1. Tocilizumab also reduced the secretion of the pro-inflammatory cytokines IL1β, IL6, TNFα, GM-CSF, IFNγ, and IL17A by Group 1 PBMCs. Finally, sALS patients had significantly higher concentrations of IL6, sIL6R and C-reactive protein in the cerebrospinal fluid when compared to AD patients. This pilot study demonstrates that *in vitro* tocilizumab suppresses many factors that drive inflammation in sALS patients, with possible increased efficacy in Group 1 ALS patients.

Keywords: Tocilizumab, amyotrophic lateral sclerosis (ALS), chronic inflammation, IL6 receptor signaling

Introduction

Neuronal degeneration in the amyotrophic lateral sclerosis (ALS) spinal cord is associated with chronic inflammation and is marked by infiltrating IL1β-, IL6-, and TNFα-positive macrophages/microglia [1] [2], as well as IL17A-positive CD8 and mast cells [3]. The inflammatory cytokines IL1β, IL6, TNFα, GM-CSF, and the bi-functional cytokine IL10, are all induced in the peripheral blood mononuclear cells (PBMCs) of ALS patients by stimulation with demetallated (Apo) or fibrillar wild-type SOD1 [4]. Mutant forms of SOD1 appear to activate the inflammation in monocytes/macrophages through activation of cyclooxygenase-2 (PTGS2 or COX-2) and caspase-1 [5]. Inflammatory macrophages, expressing IL6 and TNF-α, have been observed to phagocytize both apoptotic and non-apoptotic neurons in the spinal cord [4], suggesting a po-

tential immune mechanism that promotes neuronal death in ALS. Systemic inflammation has also been observed in early stages of the disease in ALS mouse models [6] [7].

Interleukin-6, together with the cytokine TGFβ, are well-known to promote the development of Th17 cells [8], which support chronic inflammation in autoimmune diseases [9]. IL6 is a bifunctional cytokine with both pro-inflammatory and anti-inflammatory activities: (a) "classical" signaling through the membrane-bound IL6 receptor (IL6R) for neuroprotective activities, and (b) "*trans*-signaling" by a complex formed between a soluble IL6 receptor (sIL6R) and IL6 for pro-inflammatory activities. The sIL6R-IL6 complex allows IL6 signaling in cells lacking IL6R by binding to the signaling IL6 co-receptor gp130 (gp130R) [10]. Soluble IL6R is present in serum, urine, synovial fluid, and cerebrospinal fluid (CSF) of normal subjects and is increased in subjects with autoimmune diseases [11]. IL6/sIL6R *trans*-signaling has been shown to stimulate chronic inflammation in rheumatoid joints [12].

The role of IL6 signaling in neurological diseases is not clear [10]. For example, Alzheimer's disease (AD) patients had high plasma levels of IL6, TNFα, and IL1β [13] and an increased CSF level of IL6 [14]. The CSF levels of sIL6R were found to be decreased in one study [15], but in another study equally elevated CSF concentrations of sIL6R (mean 1,000 pg/ml) in both AD patients and normal subjects were observed [16]. Accordingly, the roles of the IL6/sIL6R *trans*-signaling in the neurodegenerative diseases may vary in different stages of the disease, and may depend upon the ratios of free IL6 and sIL6R or other factors specific to each disease [17].

The IL6R antibody called tocilizumab (Actemra^{R}) inhibits IL6 signaling through both IL6R and sIL6R. Tocilizumab has shown favorable long-term effects in patients with rheumatoid arthritis [18] [19] [20], and is under study in patients with Castleman's disease, juvenile rheumatoid arthritis, and inflammatory bowel disease [21] [22]. In this study, we investigated PBMCs of ALS patients and controls regarding changes in the transcription of inflammatory cytokines, chemokines and their cognate receptors, as well as other genes; and the effect of tocilizumab on the transcription and/or secretion of cytokines and chemokines. We also showed the evidence of IL6-related inflammation in the ALS spinal cord and the cerebrospinal fluid (CSF) by testing the IL6, sIL6R, C-reactive protein (CRP) and IL1 receptor antagonist (IL1RA) levels.

Materials and methods

Study population

Eight patients with the sporadic ALS (sALS) diagnosis, 4 normal controls and one unaffected twin of an sALS patient were enrolled into the study according to the UCLA IRB approved protocol. PBMC and macrophage cultures were done as previously described.

Cytokine assays in fluids by multiplex Immunoassays

PBMCs were separated from heparin-anticoagulated blood by Ficoll-Hypaque gradient centrifugation. From each subject five million PBMCs per tube (treatment) were cultured overnight with or without 2 μg/ml of SOD1 protein. After 16-18 hours, supernatants were collected from each tube and frozen immediately for future batch testing of cytokines. The supernatant of each patient was tested with R&D Systems High Sensitivity Human Inflammation Multiplex-Kit – Pre-mixed 12- human cytokines using Luminex platform of Bio-RAD BioPlex 200 dual laser, flow-based sorting and detection analyzer. This multi-plex kit simultaneously quantified supernatant concentrations of human IL-1ß, interleukin-2 (IL2), interleukin-4 (IL4), interleukin-5 (IL5), IL6, interleukin-7 (IL7), interleukin-8 (IL8), interleukin-10 (IL10), interleukin-12 (IL12), IL13, interferon-γ (IFN□), granulocyte-macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor-α (TNFα). The results are presented in pg/ml.

RT PCR assay of inflammatory and autoimmune genes

Following isolation by the Ficoll-Hypaque technique, approximately 4.0 x 10^6 PBMCs were incubated overnight in IMDM medium alone (baseline), IMDM with 2 μg/ml SOD1, or IMDM with tocilizumab (2 μg/ml) and SOD1. Cells were pelleted by centrifugation and resuspended in RNA preserve medium (Qiagen, Valencia, CA). Total RNA was extracted using the

Table 1. ALS subjects. Subjects' age, sex, duration of illness, and FRS (functional rating scale) are listed in the table. The four control subjects for this study consisted of three UCLA professors (age > 60) and one control subject from the UCLA translational research facility.

Patient #	Disease Duration (mos)	FRS score	FRS Change/mo	Investigational Drugs /nutrition	Baseline inflammation
1	30	27	0.7* 0.17**	NP001 (placebo), Actemra, Fish oil (Smartfish)	high
2	19	39	0.4	Dexpramipexole, Ceftriaxone, NP001	high
3	28	39	0.3	Dexpramipexole, CK-357	high
4	22			Nuedexta	high
5		32		Actemra, Fish oil (Smartfish)	low
6	14	30	1.3	Actemra, fish oil	low
7	51	25	0.4	Fish oil, curcumin	low
8	24	43	0.2	Nuedexta	low

* FRS change over the entire course of disease; ** Change during Actemra^R infusion therapy (6 mo. 5/12-10/12).

RNeasy Mini-prep (+ deoxyribonuclease step) kit and, as needed, concentrated and cleaned up (Qiagen RNeasy MinElute Cleanup kit) on the day the array was plated. cDNA was then prepared from 200 ng of total RNA using the RT^2 First Strand Kit and was added to RT-PCR reagent SYBR Green Master Mix according to manufacturer protocol (Qiagen, Valencia, CA, USA). Ten microliters of the mixture was added to each of the 384-wells of the RT^2 Profiler inflammatory and autoimmune gene array PAHS-077G (Qiagen, Valencia, CA, USA). The RT-PCR reaction was performed on the Roche LightCycler 480. Data was processed by the $\Delta\Delta C_t$ method using proprietary tools supplied by SABiosciences PCR Data Analysis Web Portal (Qiagen, Valencia, CA, USA). Each custom array included control wells of (a) the threshold cycle values for genomic DNA contamination, (b) inhibition of reverse transcription, (c) a positive PCR control, and (d) three housekeeping genes. The fold regulation for a given gene was calculated by comparing a control to a treatment group and using the $2^{(-\Delta\Delta Ct)}$ normalized with housekeeping genes and other genes on the array with recorded C_t values within 0.5 cycles across the control and treatment groups.

IL17A induction in ALS PBMCs treated with aggregated SOD1

50,000 PBMCs were cultivated for 1, 5 and 8 days in the presence of aggregated SOD1 (2 μg/ml) and ALS macrophages in 8-well chamber slides. The supernatant fluids were removed for IL17A testing and the cells were stained by immunofluorescence with anti-IL17A.

Statistical analysis

The differences between the groups were tested by t-test following the Levine test for equality of variances. Correlation between cytokines was tested by Pearson's r and Spearman's rho.

Results

Baseline transcription of inflammatory cytokines and chemokines in ALS patients and controls

We previously showed by Affymetrix microarray hybridization [3] and qRT-PCR [4] that wild-type SOD1 in the Apo (demetallated) or fibrillar forms induced significantly greater transcription of

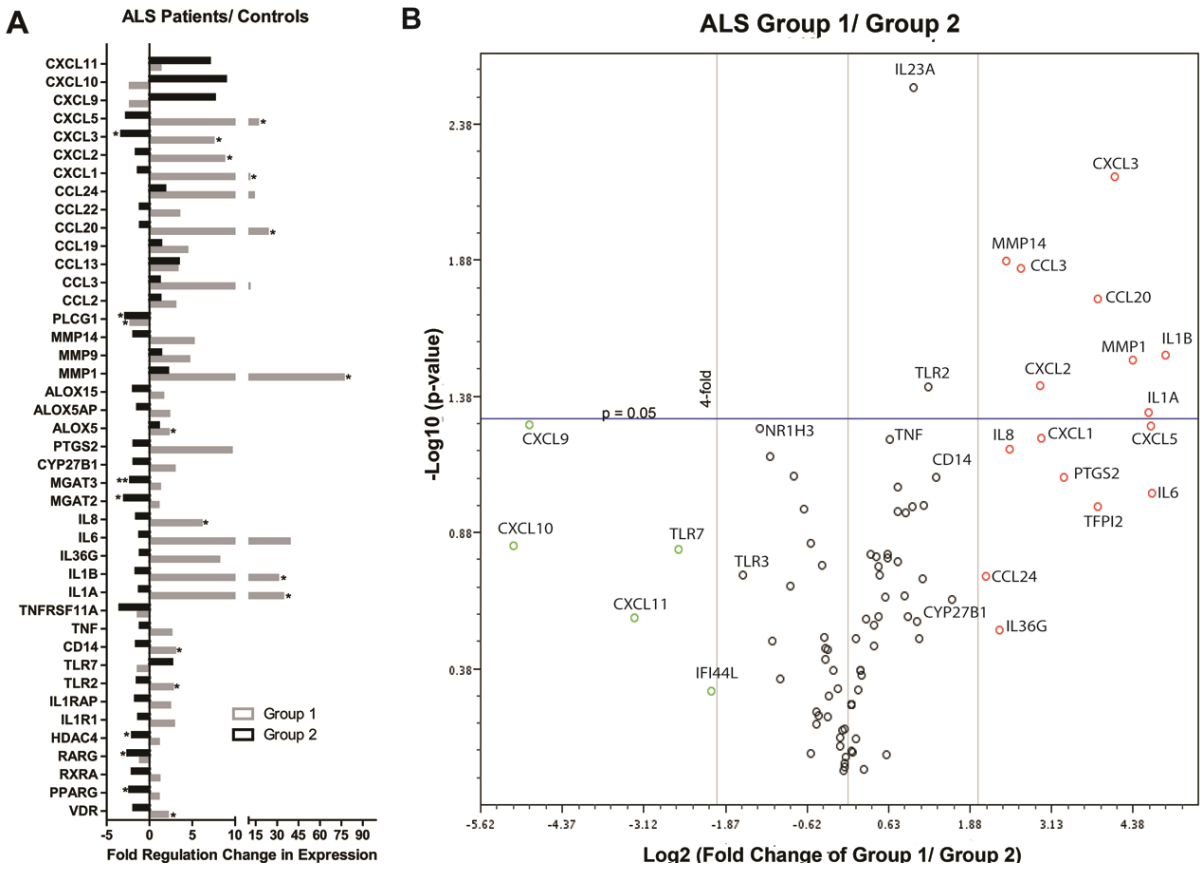

Figure 1. Baseline transcription of ninety genes associated with inflammation in the PBMCs of ALS patients compared to control subjects. **A.** The baseline expression profiles of 42 genes that were up or down regulated more than 3-fold in ALS patients when compared to controls. The patients were separated into two groups based on their expression of IL1 and IL6 with respect to the controls (see Table 1, high and low inflammation). (*) indicates that the fold regulation change was statistically significant ($p \leq 0.05$) when samples from the ALS groups (n = 7) were compared to samples from the control subjects (n = 4). (**) indicates a significant fold regulation change with $p \leq 0.01$. **B.** A volcano plot comparing the results in Group 1 with the results obtained from Group 2. Data points (i.e. mRNA expression levels) to the right of the mid-line of the plot indicate increased and to the left, decreased expression of the given gene in Group 1 patients. The two lines to the left and right of the mid-line highlight a 4-fold regulation change. The horizontal line represents a p-value of 0.05. Transcripts that were observed to be up or down regulated more than 4-fold are labeled with their gene symbols.

inflammatory cytokines and chemokines in PBMCs of sALS patients when compared to control subjects. Here we have investigated the degree of baseline (i.e. background) inflammation in PBMCs of sporadic ALS patients and controls by qRT- PCR analysis of transcription of ninety genes associated with inflammatory signaling. The PCR results suggested two groups in the study population (n=8) based on the fold regulation change in the mRNA levels of IL1 and IL6 mRNA expression when compared to the control subjects (n=4): Group 1 patients (n=4, **Table 1**) showed increased expression and Group 2 patients (n=4, **Table 1**) showed decreased or unaltered expression of IL1 and IL6.

Other genes significantly up regulated in the Group 1 PBMCs were TLR2, CD14, IL1, IL8, TNF, ALOX5, CCL3, CCL20, CXCL2, CXCL3, and CXCL5 (**Figure 1A**). Alternatively, significant down regulation of PPARg, PPARα, RARg, HDAC4, KAT2B, IL6R, IL6ST, TNFRSF11A, MGAT2, MGAT3, ADAM17, PLCG1, TSC1, TSC2 and CXCL3 was observed in the Group 2 PBMCs (**Figure 1A**). When compared to each other, Group 1 patients showed significantly higher mRNA levels of cytokines (e.g. IL1 and IL23A), chemokines (e.g. CXCL3 and CCL20), matrix

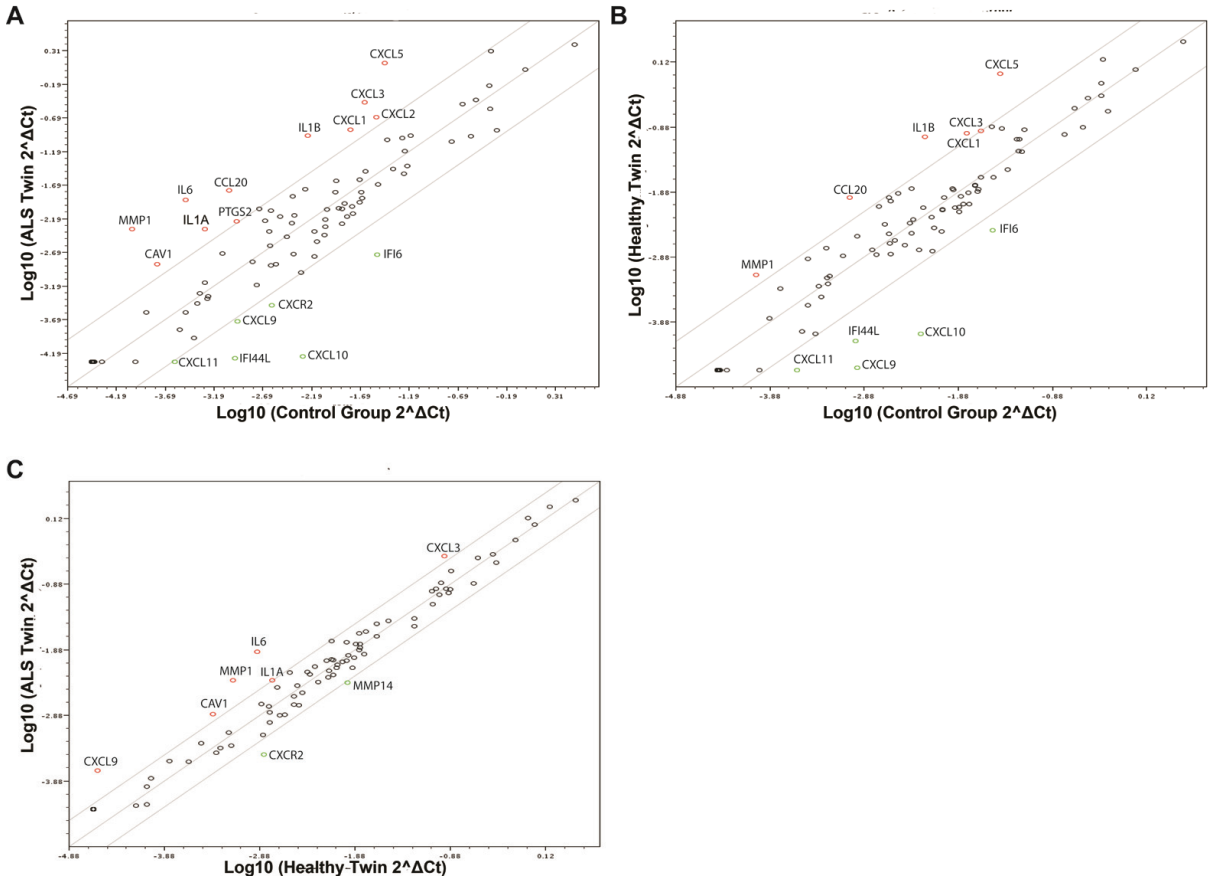

Figure 2. Baseline fold regulation changes in identical twins, one diagnosed with ALS, when compared to controls and one another. **A)** The baseline mRNA expression levels in the ALS twin (n = 2) when compared to controls (n = 4). **B)** The baseline mRNA expression levels in the unaffected identical twin (n = 2) when compared to controls (n = 4). In both panels A and B the scatter plot highlights the genes that were up or down regulated more than 4-fold when compared to controls by labeling each data point with its gene symbol. **C**) Comparison of the ALS twin with the healthy twin. Here mRNA fold regulation changes greater than 2.5-fold are indicated by labeling the data point with the gene symbol.

metalloproteinases (e.g. MMP1 and MMP14), and TLR2 than Group 2 patients (**Figure 1B**).

RT-PCR testing of PBMCs of a Group 1 ALS patient and her unaffected identical twin revealed that only the ALS patient had increased transcription of IL1α, IL6, MMP1, and CXCL3 (**Figure 2A**); however, both showed increased transcription of CXCL1, CXCL5, IL1β, and CCL20 when compared to controls (**Figures 2B** and **2C**).

Tocilizumab inhibits transcription and secretion of inflammatory cytokines induced by Apo-G37R SOD1

To test the effect of tocilizumab on IL-6/sIL6 R signaling and the downstream inflammatory genes, we first stimulated PBMCs with apo-G37R SOD1, a pathogenic form of SOD1 associated with familial ALS. As shown previously [4], apo and fibrillar forms of wild type SOD1 or apo-G37R SOD-1 stimulate IL1 and IL6 and a variety of chemokines (e.g. CCL19). Here apo-G37R SOD1 stimulated the transcription of the genes already up regulated at baseline in Group 1 and those that were similar to control subjects at baseline in Group 2 (**Table 2**). For the most part a more pronounced induction of cytokine and chemokine mRNA expression by apo-G37R was observed in Group 1 PBMCs in this pilot study. We are extending these observations in a larger sample of ALS patients.

Tocilizumab (10 μg/ml) broadly inhibited the

Table 2. Effects of exogenous apo-G37R SOD-1 and Actemra^{R} on the baseline expression of genes associated with the regulation of inflammation in ALS PBMCs

	Group 1 ALS		Group 2 ALS		Group 1/Group 2
	Effect of Apo-G37R on baseline expression	Apo-G37R + Tcz	Effect of Apo-G37R on baseline expression	Apo-G37R + Tcz	Apo-G37R compared
	Fold Regulation Change	Fold Regulation Change	Fold Regulation Change	Fold Regulation Change	Fold Regulation Change
VDR	5.15	-1.01	1.55	-1.22	2.11
PPARG	1.39	1.46	6.26	2.26	-2.35
PPARA	-3.55	-1.13	1.49	-2.69	-4.45
NR1H3	-12.7	1.45	-1.22	-1.15	-15.82
MED13	1.02	-1.02	1.09	-7.5	1.11
TLR2	5.33	2.17	2.41	1.65	1.6
IL1RAP	4.45	1.21	2.78	1.21	1.05
CD14	-47.07	1.38	1.51	-1.42	-85.83
CD163	-11.6	1.06	-1.69	-2.49	-2.72
IL1A	129.19	1.54	16.07	5.59	15
IL1B	183.97	1.33	25.75	11.42	24.88
IL36G	80.08	1.38	4.43	1.67	13.99
IL6	239.41	-2.17	17.35	7.48	20.07
IL8	17.19	1.71	13.52	7.75	4.05
IL23A	2.65	1.04	2.9	1.78	1.69
TNF	2.61	-1.04	3.29	-43.01	2
MGAT2	-1.46	-1.72	-2.09	-13.8	6.71
MGAT3	-3.47	-1.27	-6.42	-2	1.98
MGAT4A	1.21	1.44	-1.16	-4.55	-1.21
LYZ	-9.69	-1.09	-1.96	-10.6	-7.96
PDIA3	-1.24	1.42	-1.22	-233.06	-1.29
PTGS2	36.84	-1.05	13.71	7.43	3.94
ALOX15	-6.48	-5.46	1.61	1.38	-2.18
MMP1	36.93	-1.04	15.96	1.67	2.69
MMP14	6.93	-2.22	1.98	1.21	5.53
PTEN	-2.69	-1.14	-1.27	-180.6	-2.24
PLCG1	-1.68	-1.96	-1.41	-4.55	1.46
CTSD	-9.36	-2.27	-1.65	-2.08	-1.94
TFPI2	34.86	2.01	14.69	6.29	5.41
CAV1	19.2	1.62	4.85	1.46	-7.13
CCL2	1.79	1.99	3.05	-35.18	1.35
CCL3	16.83	1.33	3.22	3.04	18.59
CCL7	1.53	1.64	4.07	2.19	-1.63
CCL19	112.47	1.68	35.42	19.61	2.01
CCL20	221.83	3.23	54.07	29.72	3.78
CCL22	93.27	1.92	1.51	1.3	1.53
CCL24	4.64	3.18	-1.25	-1.76	2.14
CXCL1	19.34	2.23	22.42	8.71	6.85
CXCL2	20.87	2.58	12.1	7.64	6.01
CXCL3	13.77	1.68	13.06	-5.72	7.24
CXCL5	33.44	3.29	73.35	-243.31	1.7
CXCL9	-17.23	1.69	-20.58	-7.09	-3.26
CXCL10	-3.47	-1.74	-2.06	-1.59	1.58
CXCL11	-4.62	-2.22	-1.73	-2.08	1.54
B2M	-1.08	1.36	1.05	1.3	-1.59
RPL13A	1.46	-1.03	1.07	1	1.54
GADPH	-1.34	-1.32	-1.12	-1.3	1.03

The table summarizes four preliminary case reports; genes that showed a greater than 4-fold regulation change in the mRNA levels of group 1 and/or group 2 ALS patients (n = 2 for each group) following overnight treatment with apo-G37R (2 μg/ml) and with apo -G37R + Actemra^{R} (10 μg/ml) when compared to each patients baseline expression of the indicated genes. The right most column shows the fold regulation change values obtained when comparing the Group 1 to Group 2 apo-G37R treated PBMCs. Increased mRNA expression is denoted by a positive fold regulation change value (red) and down regulation, a negative value (blue). All three housekeeping genes on the custom RT^{2} Profiler array (Qiagen) were used for data normalization.

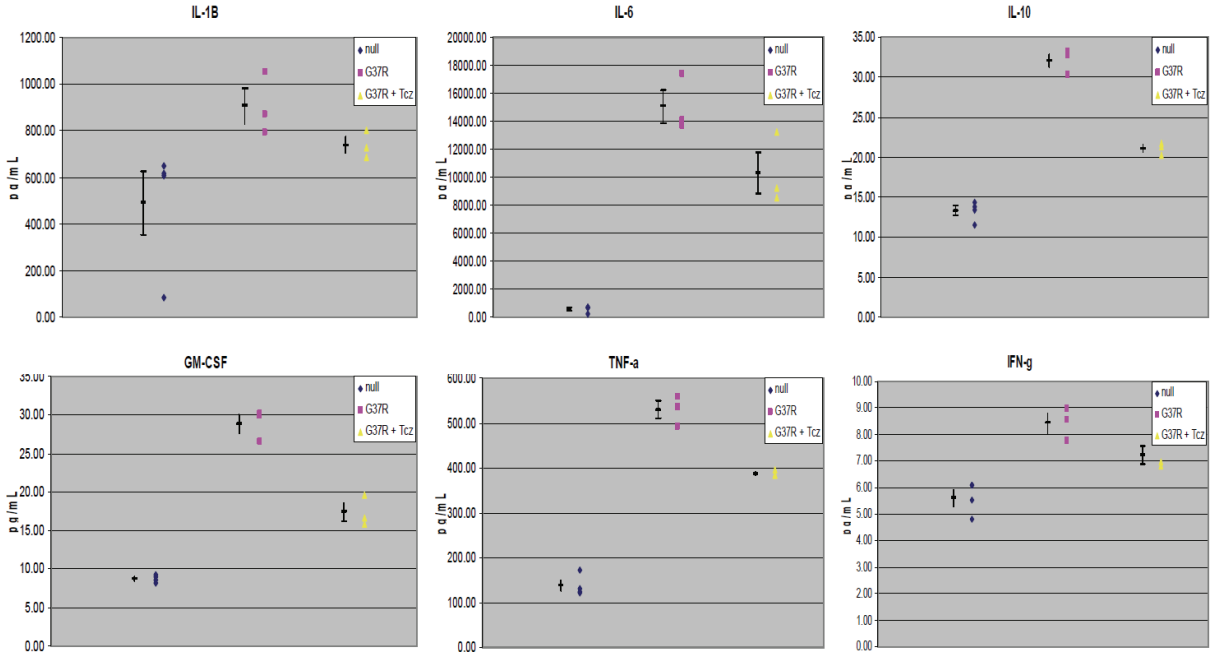

Figure 3. Tocilizumab reduces the secretion of the inflammatory cytokines in vitro. In ALS group 1 patients the concentration (pg/ml) of IL1β, IL6, IL1α, GM-CSF, TNFα and IFNγ were measured in the supernatants of PBMCs treated overnight with vehicle (null), 2 μg/ml apo-G37R or 2 μg/ml apo-G37R + 10 μg/ml tocilizumab (Tcz).

transcription of many cytokines (e.g. IL1 and IL6), some chemokines (e.g. CCL19 and CCL20) and other factors previously shown to be up regulated by apo-G37R SOD1 in sALS in comparison to control PBMCs (e.g. TFPI2). Tocilizumab had greater inhibitory potency in Group 1 than Group 2 PBMCs, as shown by the stronger return of the mRNA expression level of these genes to baseline level in Group 1 (**Table 2**). In both ALS groups tocilizumab up regulated the expression of CXCL9 and had regulatory effects on other genes (e.g. CD14, MGATs, ALOXs, MMPs etc., **Table 2**).

In addition to transcriptional effects, tocilizumab inhibited the secretion of IL1β, IL6, IL10, GM-CSF and TNFα, all of which were increased by stimulation of ALS PBMCs by apo G37R SOD-1 (**Figure 3**). Because IL17A is the cytokine involved in chronic autoimmune diseases and has been shown to be intermittently elevated in ALS patients [3], we tested the effect tocilizumab on the expression and secretion of IL17A. As shown previously, stimulation of PBMCs from a control subject co-cultured with macrophages from an sALS patient and with apo-G37R SOD1 elicits production of IL17A [4]. Here we induced cellular expression of IL17A (**Figure 4A**) and secretion of IL17A (**Figure 4B**) when the macrophages of an ALS Group 1 patient were co-cultured with control PBMCs. Both the expression and the secretion of IL17A were attenuated by tocilizumab (**Figure 4**).

Chronic inflammation in the ALS spinal cord with high levels of IL6, sIL6 receptor and C-reactive protein in the cerebrospinal fluid (CSF)

The ALS spinal cord is infiltrated by macrophages expressing IL6 and TNFα and by IL17A positive T cells and mast cells in the grey matter [3, 4]. To evaluate the degree of inflammation in CSF, we measured in the CSF the levels of the inflammatory cytokines and receptors IL6, sIL6R, C-reactive protein and IL1RA, and compared these to the CSF levels of Alzheimer disease (AD) patients. IL6 and sIL6R were found in the CSF of ALS patients in significantly higher concentrations than in the CSF of AD patients ($P = 0.008$) (**Figure 5A**). Increased sIL6R correlated with IL6 ($r=0.588$, $P= 0.057$) and IL1RA correlated with CRP ($r= 0.644$, $P=0.019$, **Figure 5B**).

Discussion

The inflammation in the ALS spinal cord and brain has been in the forefront of discussions

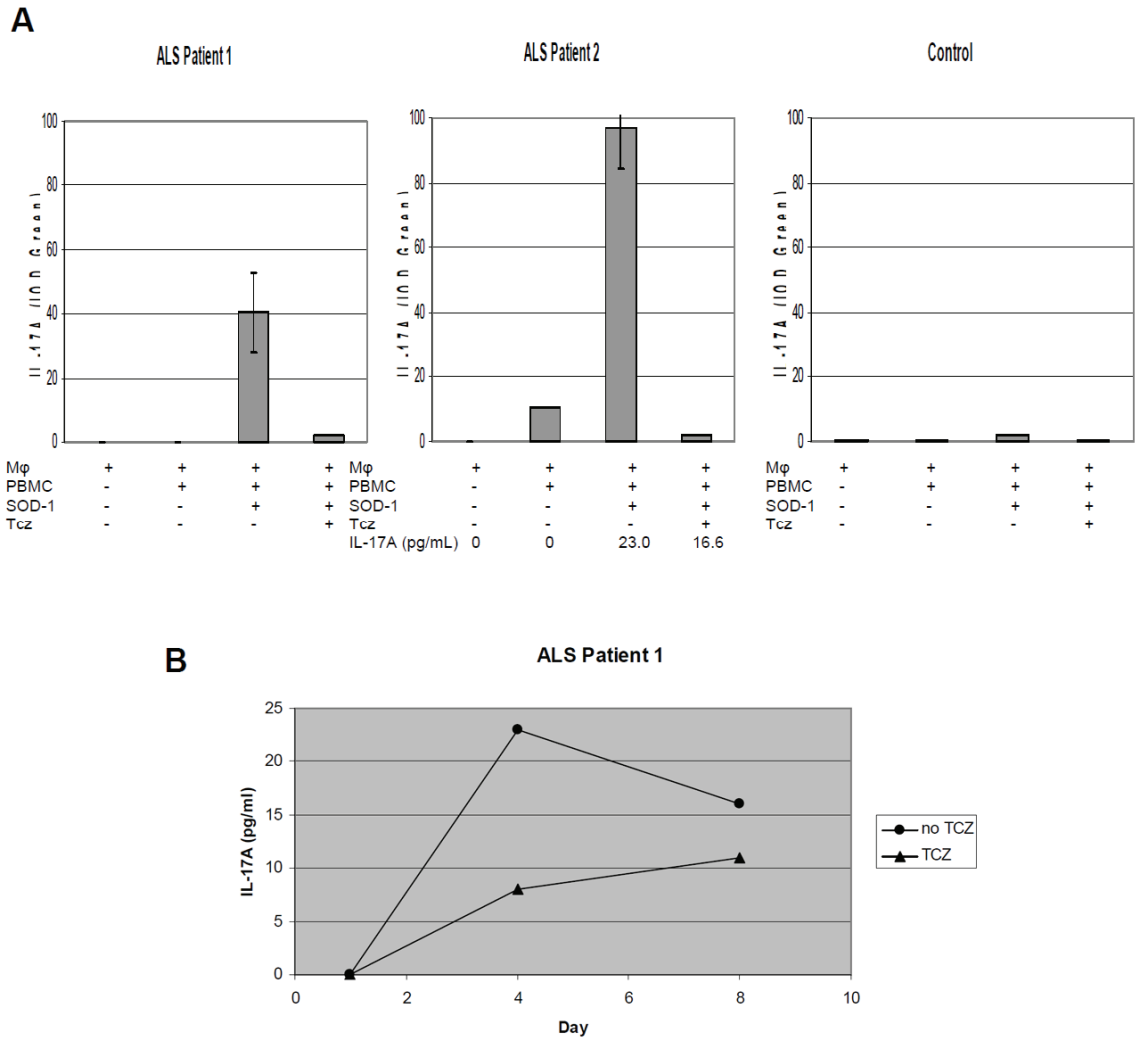

Figure 4. Tocilizumab inhibits macrophage expression and secretion of IL17A. **A.** IL17A expression on normal PBMCs co-cultured with ALS macrophages for 5 days (see methods for details) was determined by fluorescence microscopy (IOD Green/ cell). The three panels show independent results obtained with the macrophages from two ALS patients and one control. **B.** Secretion of IL17A from normal PBMCs co-cultured with ALS macrophages was monitored over an 8 day period and protein levels were determined by ELISA.

regarding ALS pathogenesis and therapy for at least 20 years [1]. Here we provide additional evidence of inflammation in the PBMCs and the CSF of ALS patients, although not all ALS patients show signs of systemic inflammation. Therefore, we separated the patients into two groups: Group 1 with high transcriptional activation and Group 2 with minor or no transcriptional activation with respect to controls. Our results in this pilot study do not clarify whether these groups reflect patients in different stages of the disease or indicate different course in patients belonging to one or the other group.

The important therapeutic objective was to investigate the effect of tocilizumab on inflammation in ALS patients. We demonstrated that tocilizumab attenuates the expression and secretion of inflammatory cytokines and chemokines in ALS PBMCs, more so in Group 1 compared to Group 2 patients.

The results of transcriptional testing showed crucial importance of IL1, IL6, MMP1, and cer-

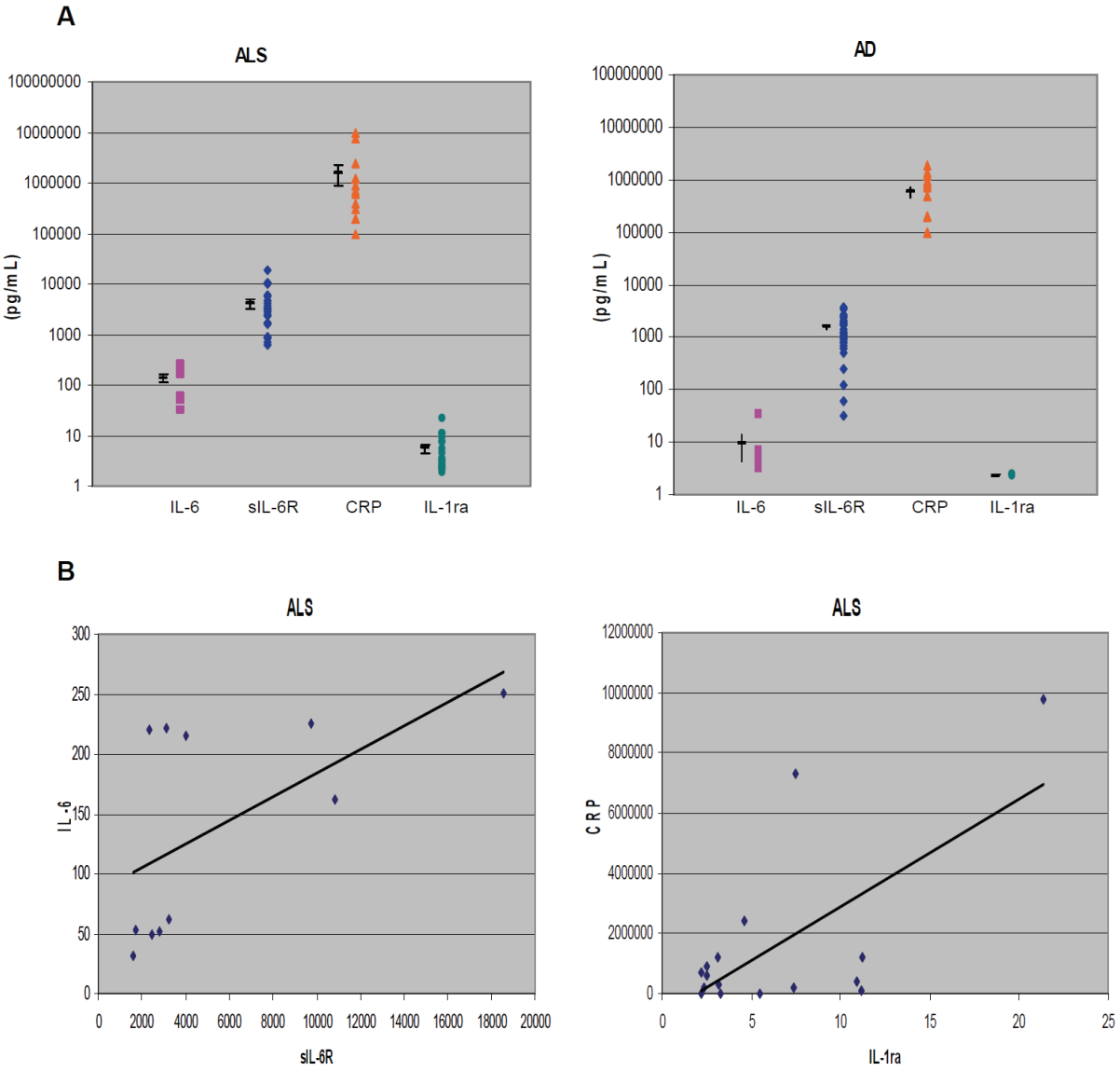

Figure 5. Protein levels (pg/ml) of IL6, sIL6R, CRP and IL1RA in the CSF of ALS and AD patients. **A.** IL6 and sIL6R were found in the CSF of post mortem ALS patients (n = 13) and AD patients (n = 13; P = 0.008). **B.** Increased IL1RA correlated with CRP (r= 0.644, P=0.019).

tain chemokines in the progression of ALS. A comparison of a pair of twins, one diagnosed with ALS and the other unaffected, indicates similarities in the up regulation of the chemokines CCL20, CXCL1 and CXCL5 and the cytokine IL1β when compared to controls. However, the ALS twin showed increased expression of MMP1, CXCL3, IL1α, and IL6 in comparison to her unaffected twin. MMP1, CXCL3, IL1α, and IL6 were up regulated in ALS PBMCs following stimulation with aggregated SOD1 in both sALS groups, as previously shown with other sALS patients by RT PCR [4] and by microarray [3]. Collectively, the RT-PCR results suggest that Group 1, but less so Group 2, patients have indicators of systemic inflammation. However, when presented with pathogenic SOD-1, a robust increase in the expression of chemokines, cytokines and activation of macrophages is observed in both Group 1 and Group 2 patients.

Tocilizumab had powerful effects on inflammation in ALS PBMCs stimulated by apo-G37R SOD1. In PBMCs of patients, tocilizumab inhib-

ited both the transcription and secretion of cytokines and chemokines induced by apo-G37R. Of note, IL1, IL6, IL36G, IL8, IL23A, TNF, PTGS2, MMP1, TFPI2, CCL7, CCL19, CCL20, CXCL1, CXCL2, CXCL3 and CXCL5 were all down regulated by tocilizumab in both ALS patient groups. Tocilizumab also inhibited the expression and secretion of the cytokine IL17A induced by ALS macrophages in co-culture with healthy PBMCs. Thus macrophages may contribute to the chronic nature of inflammation in ALS patients [3, 4] and the level of inflammation could be regulated by tocilizumab. The significance of inflammation in the ALS central nervous system was highlighted by demonstration of very high levels of IL6, sIL6 receptor and C-reactive protein in the CSF of ALS patients.

In conclusion, tocilizumab showed excellent activity against inflammatory activation in PBMCs of sALS patients in vitro. Further in vitro and in vivo studies are warranted to determine the anti-inflammatory effects of tozilizumab and potential clinical benefits of tocilizumab in sALS patients.

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Declaration of conflicts of interest,

The authors have no conflict of interest.

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