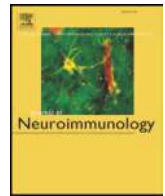




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Short communication

## Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS)

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### ABSTRACT

The present study reports elevated levels of endotoxin/lipopolysaccharide (LPS) concentrations in plasma from patients with sporadic amyotrophic lateral sclerosis (sALS) and Alzheimer's (AD) as compared to healthy controls. Levels of plasma LPS showed a significant positive correlation with degree of blood monocyte/macrophage activation in disease groups and was most elevated in patients with advanced sALS disease. There was a significant negative relationship between plasma LPS and levels of monocyte/macrophage IL-10 expression in sALS blood. These data suggest that systemic LPS levels and activated monocyte/macrophages may play significant roles in the pathogenesis of sALS.

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### 1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disorder characterized by the progressive loss of anterior-lateral horn spinal cord motor neurons leading to weakness and the eventual death of affected individuals. Although inflammatory mechanisms and immune activation have been considered as common components of the pathogenesis of ALS (Alexianu et al., 2001; Graves et al., 2004; Henkel et al., 2004; McGeer and McGeer, 2002; Simpson et al., 2004), the relevance of these processes to pathogenesis is unknown.

Endotoxin/lipopolysaccharide (LPS), is a potent inflammatory stimulus and immunostimulatory product (Takeda et al., 2003) and induces its effects through stimulation of CD14-bearing inflammatory cells (Flo et al., 2000; Tobias et al., 1999). LPS associated toxicity is mediated through systemic monocyte/macrophage and endothelial cell activation, and release of inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6) (Beutler et al., 1985; Danner et al., 1991; Okusawa et al., 1988; Tracey et al., 1986). The effects of LPS are mediated primarily through Toll-Like Receptor 4 (TLR4).

Increased levels of proinflammatory cytokines, such as MCP-1 (Baron et al., 2005; Henkel et al., 2004; Simpson et al., 2004; Wilms et al., 2003; Zhang et al., 2006), and IL-6 (Ono et al., 2001; Sekizawa et al., 1998) have been reported in cerebral spinal fluid (CSF) and sera

in patients with ALS. More recently, elevated levels of TNF- $\alpha$  have been observed in the blood of ALS patients (Babu et al., 2008; Cereda et al., 2008; Poloni et al., 2000). Our previous studies on blood specimens from patients with sporadic ALS (sALS) found elevated levels of abnormally activated monocyte/macrophages as compared to controls (Zhang et al., 2005). These data taken together suggested a potential role for LPS as a systemic monocyte activator in the pathogenesis of ALS. Therefore, the objectives of this study were: 1) to quantify the levels of plasma LPS in patients with sALS as compared to control groups, 2) to test whether levels of plasma LPS would correlate with monocyte activation and/or monocyte IL-10 (a regulator of activation) expression in blood, and 3) to determine if LPS levels in plasma correlated with clinical stage of disease in sALS.

### 2. Materials and methods

#### 2.1. Subjects

Twenty-three patients diagnosed with sALS (7 females and 16 males, mean age  $59.2 \pm 8.7$  years) by El Escorial criteria (Brooks, 1994) at the Forbes Norris MDA/ALS Research Center (San Francisco, California, USA) had blood drawn in accordance with the CPMC (California Pacific Medical Center) and UCSF committees on human research guidelines, coordinated by the UCSF AIDS and Cancer Specimen Resource (ACSR) program and excluded patients with any evidence for intercurrent infection. The Revised ALS Functional Rating Scale (ALSFRS-R), scored 0–48, was used to evaluate overall patient functional status (Cedarbaum et al., 1999). All scores were updated within a month of blood testing. Demographic and medication

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information for sALS patients whose specimens were studied are shown in Table 1.

Two control groups were used in the study. The first control group consisted of 18 age-matched healthy donors (6 females and 12 males, mean age  $54.5 \pm 8.4$  years, Stanford University Blood Center). The second control group obtained from the memory clinic at Forbes Norris Center, had Alzheimer's disease (AD). This group (11 females and 7 males, mean age  $78.9 \pm 8.3$  years) was used as neurological disease controls and had no active infection at the time of blood donation into the study. All control blood specimens were processed in a similar manner to the sALS patient blood specimens.

## 2.2. Flow cytometry

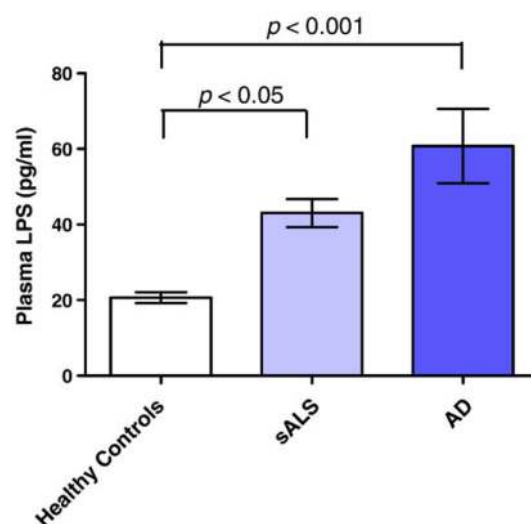
Blood immunophenotyping studies were performed as previously described (Zhang et al., 2005). Monocyte activation was evaluated by quantitating levels of HLA-DR on CD14 cells. IL-10 expression in CD14 cells was used as another marker for its function as a regulator of monocyte/macrophage activation.

## 2.3. Endotoxin detection

Plasma from sALS, AD and healthy controls blood was obtained by Percoll gradient centrifugation, and was frozen at  $-70^\circ\text{C}$  until assayed. Duplicate plasma LPS levels in all plasma specimens were quantified by the LAL (Limulus Amebocyte Lysate) Chromogenic Endpoint Assay (Cell Sciences Inc., Canton, MA, USA) according to the manufacturer's instructions.

## 2.4. Statistical analysis

Cut-off values for defining cell activation as "positive" and "negative" for sALS patients and disease controls were determined by comparison with values from healthy donors. Results are expressed as the mean  $\pm$  SD. Statistical analysis of group distribution and differences, linear regression, and Pearson correlations were performed by GraphPad Prism 4.0 program (GraphPad Software, San



**Fig. 1.** Plasma levels of LPS in healthy controls ( $21 \pm 6$  pg/ml,  $n=18$ ), AD ( $61 \pm 42$  pg/ml,  $n=18$ ), and sALS ( $43 \pm 18$  pg/ml,  $n=23$ ).

Diego, California, USA). Between-group comparisons were made using One-Way ANOVA with Tukey's multiple comparison tests. For all analyses, a value of  $p < 0.05$  was considered statistically significant.

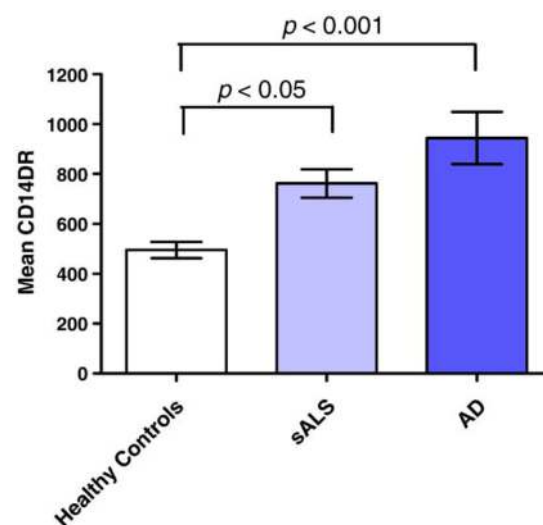
## 3. Results

### 3.1. Plasma endotoxin LPS levels

Compared to the healthy population, significantly higher levels of LPS were observed in sALS ( $p < 0.05$ ) and AD ( $p < 0.001$ ) plasma specimens (Fig. 1). Plasma levels of LPS were similar between sALS and AD disease groups.

### 3.2. Monocyte activation and plasma LPS levels

The levels of monocyte activation marker HLA-DR detected on sALS ( $p < 0.05$ ) and AD ( $p < 0.001$ ) were significantly higher than that detected on normal blood monocytes (Fig. 2) and were directly related to the level of plasma LPS. Fig. 3 shows that monocyte HLA-DR varied



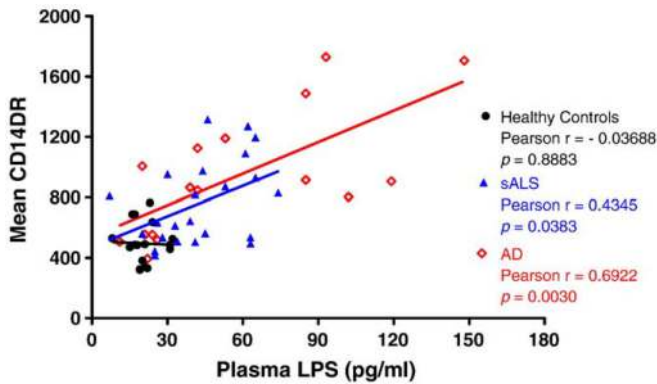
**Fig. 2.** Degree of monocyte HLA-DR expression in healthy controls (mean  $\text{CD14DR} = 495 \pm 136$ ,  $n=17$ ), AD (mean  $\text{CD14DR} = 943 \pm 417$ ,  $n=17$ ), and sALS (mean  $\text{CD14DR} = 761 \pm 274$ ,  $n=23$ ).

**Table 1**  
Clinical summary of sALS patients

Patient ID	Patient age (years)	Patient gender	Duration of illness (months)	ALSFRS-R score	Therapy	
					Riluzole <sup>a</sup>	NSAIDs <sup>b</sup>
Patient 1	52	M	46	34	Yes	No
Patient 2	51	F	19	39	Yes	No
Patient 3	63	M	24	19	Yes	No
Patient 4	66	M	17	39	Yes	Yes
Patient 5	48	F	8	33	Yes	No
Patient 6	72	M	17	37	Yes	No
Patient 7	45	M	16	33	Yes	No
Patient 8	52	F	40	29	Yes	Yes
Patient 9	54	M	9	45	Yes	Yes
Patient 10	52	F	24	29	Yes	No
Patient 11	70	M	17	36	Yes	Yes
Patient 12	52	M	8	38	Yes	Yes
Patient 13	59	M	11	42	Yes	No
Patient 14	70	M	12	35	Yes	Yes
Patient 15	53	M	18	35	Yes	No
Patient 16	73	F	21	34	Yes	No
Patient 17	62	M	26	33	Yes	Yes
Patient 18	52	F	20	42	Yes	No
Patient 19	74	M	29	31	No	Yes
Patient 20	63	M	6	37	No	No
Patient 21	66	M	21	34	Yes	Yes
Patient 22	54	M	22	37	Yes	No
Patient 23	58	F	7	37	Yes	Yes

<sup>a</sup> Fifty milligrams twice daily.

<sup>b</sup> NSAIDs, non-steroidal anti-inflammatory drugs (Aspirin, Ibuprofen, Motrin), standard dose.



**Fig. 3.** Correlations between monocyte activation and plasma LPS levels in healthy controls, AD, and sALS: significantly positive correlation of the degree of monocyte HLA-DR expression with levels of plasma LPS in both sALS and AD.

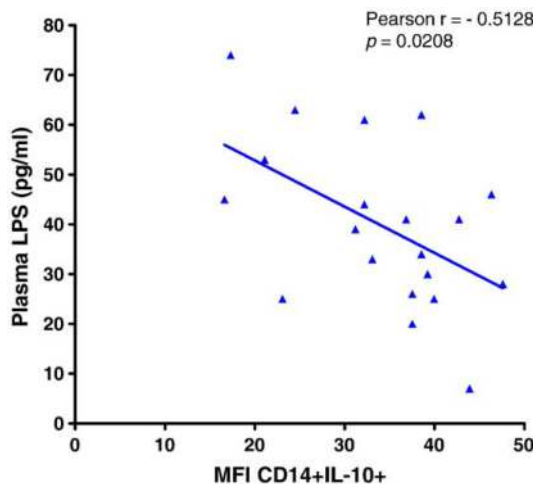
in a direct relationship to plasma LPS levels in both sALS ( $r=0.4345$ ,  $p=0.0383$ ) and AD ( $r=0.6922$ ,  $p=0.0030$ ) groups. No correlation was found between degree of monocyte activation and plasma LPS levels in healthy group.

### 3.3. Loss of monocyte IL-10 expression and plasma LPS levels in sALS

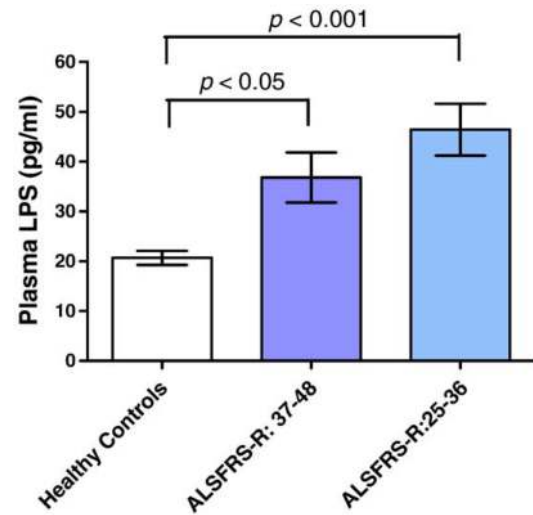
The levels of monocyte IL-10 expression (Median Fluorescence Intensity) were significantly higher in AD ( $49 \pm 26$ ,  $n=17$ ) compared to sALS ( $34 \pm 9$ ,  $n=20$ ,  $p<0.05$ ) and healthy controls ( $33 \pm 11$ ,  $n=17$ ,  $p<0.05$ ). No significant differences in monocyte IL-10 expression were observed between sALS and healthy controls. However, there was a significant inverse correlation between plasma LPS levels and degree of CD14 cell IL-10 expression in sALS blood ( $r=-0.5128$ ,  $p=0.0208$ ), as shown in Fig. 4, whereas no correlation was found between monocyte IL-10 expression and plasma LPS levels in AD and healthy controls (data not shown).

### 3.4. Plasma LPS levels and clinical disease status in sALS

To evaluate whether plasma LPS levels would be related to severity of disease, the plasma LPS results of patients with sALS from Fig. 1 were compared with the clinical ALS values shown in Table 1. This analysis was performed by dividing sALS patients into two groups based on ALSFRS-R quartile scores. Those with moderate impairment (an ALSFRS-R score of 25–36,  $n=12$ ) were compared to those with early impairment (an ALSFRS-R score of 37–48,  $n=10$ ). As shown in Fig. 5,



**Fig. 4.** Relationship of monocyte IL-10 expression to plasma LPS levels in sALS: negative correlation of degree of IL-10 expression on CD14 cells with plasma LPS levels in sALS.



**Fig. 5.** Relationship of ALSFRS-R scores to plasma LPS levels in sALS patients. Increased LPS levels were highly significant in patients with moderate impairment ( $n=12$ ,  $p<0.001$ ) compared to healthy controls ( $n=18$ ).

increased levels of plasma LPS were highly significant in patients with moderate impairment as compared with healthy controls ( $p<0.001$ ).

## 4. Discussion

In the current study, we show for the first time that plasma levels of LPS are elevated in patients with sALS and AD. Plasma LPS levels may also be related to clinical disease status in sALS. Early stage sALS patients had lower levels of plasma LPS than did patients with more advanced disease. Elevated levels of abnormally activated monocyte/macrophages defined by CD14 co-expression of HLA-DR were found in patients with sALS and AD both in the current and in our previous study (Zhang et al., 2005). Monocyte activation levels in the current study varied directly with plasma LPS levels. Moreover, the levels of plasma LPS were inversely correlated with monocyte IL-10 expression in sALS patients.

Recent studies on immune activation and disease have found that monocyte/macrophage activation in chronic HIV infection/AIDS (Brenchley et al., 2006) is associated with LPS levels from gut associated microbial translocation. Sources of plasma LPS include, but are not limited to, commensal and pathogenic bacteria, as well as subclinical opportunistic infections. None of our patients had any active infection at the time of the study. It seems that plasma LPS in sALS and AD might also be from gut associated microbial translocation like that observed in the chronic HIV infection (Brenchley et al., 2006). As a systemic macrophage activator, LPS administration leads to acute neuronal cell death (Cunningham et al., 2005) and chronic neuroinflammation and progressive neurodegeneration (Qin et al., 2007). More specific to ALS, the injection of LPS into SOD1(G37R) ALS mice (Nguyen et al., 2004) caused a dramatic shortening of their lifespan suggesting that LPS-mediated macrophage activation may exacerbate the pathogenesis of ALS in vivo. The current study suggests that circulating LPS, without specifying the source of LPS, may contribute to disease development in sALS.

Whereas LPS induces classical monocyte activation and production of inflammatory mediators, IL-10 is a cytokine with anti-inflammatory properties that may downregulate monocyte activation (de Waal Malefyt et al., 1991; Ramani et al., 1993). The expression of this endogenous IL-10 conferred significant protection from the harmful effects of LPS challenge and reduced expression of pro-inflammatory cytokines such as TNF- $\alpha$ , (Gerard et al., 1993; Marchant et al., 1994) and regulated leukocyte–endothelial cell interactions, and microvascular permeability (Hickey et al., 1998). In other experimental systems,

IL-10 exhibited protective effects in models of local inflammation including brain or spinal cord injury (Bethua et al., 1999).

High LPS levels observed in plasma from patients with advanced sALS were associated with lower circulating monocyte IL-10 expression. It is unlikely that this decreased level of IL-10 was directly related to systemic exposure of monocytes to elevated levels of circulating LPS as AD patients also had high levels of plasma LPS, but no correlation was found with monocyte IL-10 expression. The loss of systemic monocyte IL-10 expression associated with high plasma LPS levels in sALS may be related to disease pathogenesis. It is possible that LPS associated IL-10 reductions may reduce the endogenous anti-inflammatory capability allowing neuroinflammatory disease progression. Accordingly, the balance between pro- and anti-inflammatory TLR4 signaling pathways triggered in response to bacterial products may influence the development of ALS disease. Systemic LPS levels and LPS activated monocyte/macrophage represent two new co-factors that may play significant roles in the pathogenesis of ALS and as such represent novel targets for therapeutic intervention in patients with ALS.

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## References

- Alexianu, M.E., Kozovska, M., Appel, S.H., 2001. Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* 57, 1282–1289.
- Babu, G.N., Kumar, A., Chandra, R., Puri, S.K., Kalita, J., Misra, U.K., 2008. Elevated inflammatory markers in a group of amyotrophic lateral sclerosis patients from northern India. *Neurochem. Res.* 33, 1145–1149.
- Baron, P., Bussini, S., Cardin, V., Corbo, M., Conti, G., Galimberti, D., Scarpini, E., Bresolin, N., Wharton, S.B., Shaw, P.J., Silani, V., 2005. Production of monocyte chemoattractant protein-1 in amyotrophic lateral sclerosis. *Muscle Nerve* 32, 541–544.
- Bethua, J.R., Nagashima, H., Acosta, M.C., Briceno, C., Gomez, F., Marcillo, A.E., Loo, K., Green, J., Dietrich, W.D., 1999. Systemically administered interleukin-10 reduces tumor necrosis factor- $\alpha$  production and significantly improves functional recovery following traumatic spinal cord injury in rats. *J. Neurotrauma* 16, 851–863.
- Beutler, B., Milsark, I.W., Cerami, A.C., 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* 229, 869–871.
- Brenchley, J.M., Price, D.A., Schacker, T.W., Asher, T.E., Silvestri, G., Rao, S., Kazzaz, Z., Bornstein, E., Lambotte, O., Altmann, D., Blazar, B.R., Rodriguez, B., Teixeira-Johnson, L., Landay, A., Martin, J.N., Hecht, F.M., Picker, L.J., Lederman, M.M., Deeks, S.G., Douek, D.C., 2006. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* 12, 1365–1371.
- Brooks, B.R., 1994. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. *J. Neurol. Sci.* 124, 96–107 Suppl.
- Cedarbaum, J.M., Stambler, N., Malta, E., Fuller, C., Hilt, D., Thurmond, B., Nakanishi, A., 1999. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J. Neurol. Sci.* 169, 13–21.
- Cereda, C., Baiocchi, C., Bongioanni, P., Cova, E., Guareschi, S., Metelli, M.R., Rossi, B., Sbalsi, I., Cuccia, M.C., Ceroni, M., 2008. TNF and sTNFR1/2 plasma levels in ALS patients. *J. Neuroimmunol.* 194, 123–131.
- Cunningham, C., Wilcockson, D.C., Campion, S., Lunnon, K., Perry, V.H., 2005. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J. Neurosci.* 25, 9275–9284.
- Danner, R.L., Elin, R.J., Hosseini, J.M., Wesley, R.A., Reilly, J.M., Parillo, J.E., 1991. Endotoxemia in human septic shock. *Chest* 99, 169–175.
- de Waal Malefyt, R., Abrams, J., Bennett, B., Figdor, C.G., de Vries, J.E., 1991. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 174, 1209–1220.
- Flo, T.H., Halaas, O., Lien, E., Ryan, L., Teti, G., Golenbock, D.T., Sundan, A., Espevik, T., 2000. Human toll-like receptor 2 mediates monocyte activation by *Listeria monocytogenes*, but not by group B streptococci or lipopolysaccharide. *J. Immunol.* 164, 2064–2069.
- Gerard, C., Bruyns, C., Marchant, A., Abramowicz, D., Vandenamee, P., Delvaux, A., Fiers, W., Goldman, M., Velu, T., 1993. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *J. Exp. Med.* 177, 547–550.
- Graves, M.C., Fiala, M., Dinglasan, L.A., Liu, N.Q., Sayre, J., Chiappelli, F., van Kooten, C., Vinters, H.V., 2004. Inflammation in amyotrophic lateral sclerosis spinal cord and brain is mediated by activated macrophages, mast cells and T cells. *Amyotroph. Lateral Scler. Other Mot. Neuron Disord.* 5, 213–219.
- Henkel, J.S., Engelhardt, J.I., Siklos, L., Simpson, E.P., Kim, S.H., Pan, T., Goodman, J.C., Siddique, T., Beers, D.R., Appel, S.H., 2004. Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. *Ann. Neurol.* 55, 221–235.
- Hickey, M.J., Issekutz, A.C., Reinhardt, P.H., Fedorak, R.N., Kubes, P., 1998. Endogenous interleukin-10 regulates hemodynamic parameters, leukocyte-endothelial cell interactions, and microvascular permeability during endotoxemia. *Circ. Res.* 83, 1124–1131.
- Marchant, A., Bruyns, C., Vandenamee, P., Abramowicz, D., Gerard, C., Delvaux, A., Ghezzi, P., Velu, T., Goldman, M., 1994. The protective role of interleukin-10 in endotoxin shock. *Prog. Clin. Biol. Res.* 388, 417–423.
- McGeer, P.L., McGeer, E.G., 2002. Inflammatory processes in amyotrophic lateral sclerosis. *Muscle Nerve* 26, 459–470.
- Nguyen, M.D., D'Aigle, T., Gowing, G., Julien, J.P., Rivest, S., 2004. Exacerbation of motor neuron disease by chronic stimulation of innate immunity in a mouse model of amyotrophic lateral sclerosis. *J. Neurosci.* 24, 1340–1349.
- Okusawa, S., Gelfand, J.A., Ikejima, T., Connolly, R.J., Dinarello, C.A., 1988. Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. *J. Clin. Invest.* 81, 1162–1172.
- Ono, S., Hu, J., Shimizu, N., Imai, T., Nakagawa, H., 2001. Increased interleukin-6 of skin and serum in amyotrophic lateral sclerosis. *J. Neurol. Sci.* 187, 27–34.
- Poloni, M., Facchetti, D., Mai, R., Micheli, A., Agnoletti, L., Francolini, G., Mora, G., Camana, C., Mazzini, L., Bachetti, T., 2000. Circulating levels of tumor necrosis factor- $\alpha$  and its soluble receptors are increased in the blood of patients with amyotrophic lateral sclerosis. *Neurosci. Lett.* 287, 211–214.
- Qin, L., Wu, X., Block, M.L., Liu, Y., Breese, G.R., Hong, J.S., Knapp, D.J., Crews, F.T., 2007. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* 55, 453–462.
- Ramani, M., Ollivier, V., Khechai, F., Vu, T., Ternisien, C., Bridey, F., de Prost, D., 1993. Interleukin-10 inhibits endotoxin-induced tissue factor mRNA production by human monocytes. *FEBS Lett.* 334, 114–116.
- Sekizawa, T., Openshaw, H., Ohbo, K., Sugamura, K., Itoyama, Y., Niland, J.C., 1998. Cerebrospinal fluid interleukin 6 in amyotrophic lateral sclerosis: immunological parameter and comparison with inflammatory and non-inflammatory central nervous system diseases. *J. Neurol. Sci.* 154, 194–199.
- Simpson, E.P., Henry, Y.K., Henkel, J.S., Smith, R.G., Appel, S.H., 2004. Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden. *Neurology* 62, 1758–1765.
- Takeda, K., Kaisho, T., Akira, S., 2003. Toll-like receptors. *Annu. Rev. Immunol.* 21, 335–376.
- Tobias, P.S., Tapping, R.I., Gegner, J.A., 1999. Endotoxin interactions with lipopolysaccharide-responsive cells. *Clin. Infect. Dis.* 28, 476–481.
- Tracey, K.J., Beutler, B., Lowry, S.F., Merryweather, J., Wolpe, S., Milsark, I.W., Hariri, R.J., Fahey, T.J., Zentella, A., Albert, J.D., et al., 1986. Shock and tissue injury induced by recombinant human cachectin. *Science* 234, 470–474.
- Wilms, H., Sievers, J., Dengler, R., Bufler, J., Deuschl, G., Lucius, R., 2003. Intrathecal synthesis of monocyte chemoattractant protein-1 (MCP-1) in amyotrophic lateral sclerosis: further evidence for microglial activation in neurodegeneration. *J. Neuroimmunol.* 144, 139–142.
- Zhang, R., Gascon, R., Miller, R.G., Gelinas, D.F., Mass, J., Hadlock, K., Jin, X., Reis, J., Narvaez, A., McGrath, M.S., 2005. Evidence for systemic immune system alterations in sporadic amyotrophic lateral sclerosis (sALS). *J. Neuroimmunol.* 159, 215–224.
- Zhang, R., Gascon, R., Miller, R.G., Gelinas, D.F., Mass, J., Lancero, M., Narvaez, A., McGrath, M.S., 2006. MCP-1 chemokine receptor CCR2 is decreased on circulating monocytes in sporadic amyotrophic lateral sclerosis (sALS). *J. Neuroimmunol.* 179, 87–93.