Serum programmed cell death proteins in amyotrophic lateral sclerosis

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A B S T R A C T

Amyotrophic lateral sclerosis (ALS) is a multifactorial, multisystem pro-inflammatory neuromuscular disorder. Activation of programmed cell death-1 (PD-1), and its ligands, programmed cell death-ligand 1 and 2 (PD-L1/L2), leads to immune suppression. Serum soluble forms of these proteins, sPD-1/sPD-L1/sPD-L2, inhibit this suppression and promote pro-inflammatory responses. The purpose of this study was to determine if sPD-1, sPD-L1, and sPD-L2 were increased in sera of patients with ALS. sPD-1 and sPD-L2 were elevated in sera of patients and accurately reflected patients’ disease burdens. Increased sera levels of programmed cell death proteins reinforce the concept that peripheral pro-inflammatory responses contribute to systemic inflammation in patients with ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common, devastating and invariably fatal adult motoneuron disease with immune activation playing a prominent role in its pathobiology (Brown and Al-Chalabi, 2017; Beers and Appel, 2019). In addition to well-known central nervous system inflammatory findings, there is emerging evidence of clinically relevant peripheral pro-inflammatory responses in these patients; ALS is a multifactorial, multisystem disease in which the central nervous and peripheral immune systems contribute to disease progression and burden (Henkel et al., 2013; Beers et al., 2017; Zhao et al., 2017; Sheean et al., 2018). Several earlier studies have reported increased numbers of pro-inflammatory lymphocytes and monocytes in patients that correlated with disease progression; a heightened systemic inflammatory state is associated with a worse prognosis in ALS (Keizman et al., 2009; Hu et al., 2017; Murdoch et al., 2017; Gantafson et al., 2017). A recent study reported that acute phase proteins (APPs), which are proteins elevated due to inflammation, were increased in sera of patients with ALS; these increased serum APP levels accurately reflected patients’ disease burdens, progression rates, and survival times, providing further evidence that ALS is a systemic pro-inflammatory disorder (Beers et al., 2020).

Immune regulatory checkpoint pathways play important roles in maintaining the homeostasis of the immune system (Obst et al., 2018). While neurologists are focused on inhibiting the central and peripheral pro-inflammatory responses, and slowing disease progression in patients with ALS, oncologists are focused on impeding these regulatory checkpoint pathways, and consequently enhancing pro-inflammatory immune responses to target and eliminate tumor cells in patients with cancer (Henkel et al., 2013; Beers et al., 2017; Thonhoff et al., 2018; Obst et al., 2018). Thus, ALS and tumor pathobiologies may be thought of as opposite ends of a detrimental versus beneficial spectrum of pro-inflammatory immune responses.

Programmed cell death-1 (PD-1) protein, and its ligands, programmed cell death-ligand 1 and 2 (PD-L1/PD-L2), three proteins involved in these regulatory pathways, are expressed on lymphocytes and monocytes, and suppress activation of these immune cells, essentially acting as a ‘brake’ on immune system activation (Gu et al., 2019). However, serum soluble forms of these proteins, sPD-1/sPD-L1/sPD-L2, inhibit this suppression (Pukasawa et al., 2017). Since chronic systemic inflammation occurs in patients with ALS, these soluble proteins may contribute to exacerbated pro-inflammatory immune responses. Therefore, this study evaluated sPD-1/sPD-L1/sPD-L2 levels in the sera of patients with ALS and correlated these levels with disease burdens.

2. Materials and methods

2.1. Patients

Standard protocol approvals, registrations, and patient consents were
obtained. This was a retrospective cohort study of patients and healthy controls (HC) from our MDA/ALSA ALS clinic at the Houston Methodist Hospital. Patients were diagnosed according to the revised El Escorial criteria and their disease burdens were determined by their Appel ALS (AALS) score (range: 30–164) (Haverkamp et al., 1995). Fast progressing patients were defined as those progressing at a rate of greater than or equal to 1.5 AALS points/month whereas slowly progressing patients progressed at less than 1.5 AALS points/month (Henkel et al., 2013). HC were typically spouses and friends of patients, and exclusion criteria included any other neurologic conditions, or autoimmune or infectious diseases. The demographics of patients with ALS ($n = 90$) were $62.6 \pm 1.47$ years old (mean $\pm$ SD); 59.0% were men and 41.0% were women; and 85.9% were white, 4.56% were Hispanic, 6.03% were black, and 3.51% were Asian. HC ($n = 30$) were similar $63.5 \pm 1.15$ years old; 45.0% were men and 55.0% were women; and 91.5% were white, 4.1% were Hispanic, none were black, and 4.09% were Asian. Patients with ALS were treated per standard of care; there are no reported effects of the FDA-approved medication Riluzole on serum levels of PD-1, PD-L1, or PD-L2.

### 2.2. Enzyme-linked immunoassays (ELISA)

Human PD-1 and PD-L2 ELISA Kits from Abcam, and a PD-L1 ELISA kit from R&D Systems, were used to determine the concentration of PD-1, PD-L1, and PD-L2 protein levels according to manufacturer’s instructions. Human soluble CD14 (sCD14) and C-reactive protein (CRP) quantikine, and lipopolysaccharide binding protein (LBP) DuoSet, ELISA Kits from R&D Systems were used to determine the concentration of sCD14, LBP, or CRP protein levels in patient sera according to manufacturer’s instructions.

### 2.3. Statistics

Comparisons were performed using ANOVA for more than 2 groups or Student’s $t$-test for two groups. The ANOVA is presented with the degrees of freedom, F value, and $p$ value. Correlation was done using Spearman Rank Order in SigmaStat software. The Spearman correlation is presented with a rho ($r$) value and a $p$ value. Student’s $t$-tests are presented with $p$ values. Data are expressed as mean $\pm$ SEM and $p$ values less than 0.05 were considered significant.

### 3. Results

#### 3.1. PD-1, PD-L1, and PD-L2 serum levels

sPD-1 levels were elevated in the sera of all patients with ALS ($n = 90$) when compared with HC ($n = 30$) ($p = 0.02$) (Table 1). When separated into fast progressing and slowly progressing patients, sPD-1 was only elevated in sera from fast progressing patients compared with HC ($p = 0.007$); there were no differences between fast and slowly progressing patients or between slowly progressing patients and HC ($F(2, 117) = 2.86, p = 0.06$). sPD-L1 was not different between patients and HC. sPD-L2 was increased in the sera of all patients with ALS compared with HC ($p < 0.001$), and in fast progressing ($p < 0.001$) and slowly progressing ($p = 0.006$) patients compared with HC ($F(2, 117) = 15.48, p < 0.001$); fast progressing patients also had elevated sera PD-L2 compared with slowly progressing patients ($p = 0.001$). Sera sPD-L2 levels in HC were 61 and 227 times more elevated than serum sPD-1 and sPD-L1, respectively.

#### 3.2. PD-1 and PD-L2 correlations with patients’ disease burdens

Sera sPD-1 and sPD-L2 positively correlated with patients’ disease burdens ($r = 0.332, p = 0.001; r = 0.582, p < 0.001$, respectively) (Fig. 1A and B); the more serum sPD-1 and PD-L2, the greater the disease burden. sPD-L1 did not correlate with patients’ disease burdens (data not shown). sPD-1 positively correlated with sPD-L2; the greater the level of serum sPD-1, the greater the level of serum sPD-L2 ($r = 0.496, p < 0.001$) (Fig. 1C).

Sera sPD-1 and sPD-L2 levels did not correlate with patients’ sera levels of sCD14 or CRP (data not shown). However, serum sPD-1 and PD-L2 levels positively correlated with sera LBP levels; the more serum sPD-1 and PD-L2, the greater the levels of LBP ($r = 0.360, p < 0.001; r = 0.475, p < 0.001$, respectively) (Fig. 1D and E).

### 4. Discussion

Previous studies have demonstrated that as ALS progresses and disease burden escalates, the cascade of pro-inflammatory responses are concomitantly increased. (Beers et al. 2017, 2020). However, in contrast with ALS, studies in cancer biology have shown that tumors have developed exquisite mechanisms to facilitate escape from immune surveillance by creating an immunosuppressive microenvironment either locally at the site of the tumor or systemically. The interaction of PD-1 and its cognate ligands, PD-L1 and PD-L2, are immune checkpoint proteins that induce this suppressive modulation and have essential roles in balancing protective immunity and immunopathology, homeostasis and tolerance; the interactions between these proteins are not only important for maintaining immune tolerance but also for providing the means through which tumors evade the immune system. Upon activation, these co-inhibitory ‘checkpoints’ become induced to regulate T lymphocytes (Gu et al., 2018). However, during responses to chronic disorders, the expression of these proteins can limit protective immunity.

Numerous co-stimulatory molecules in immunoregulation pathways assume two forms of expression, namely membrane-bound and soluble.

Table 1

|                      | All (A) ALS Patients | Slow (S) progressing ALS Patients | Fast (F) progressing ALS Patients | Healthy Controls (HC) | $p$ values (Student’s $t$ or ANOVA) |
|----------------------|----------------------|-----------------------------------|-----------------------------------|-----------------------|----------------------------------|
| sPD-1 (pg/ml)        | 220.72 ± 7.90        | 210.70 ± 10.99                    | 232.69 ± 10.24                    | 194.13 ± 8.06         | A vs. HC = 0.02                  |
|                      |                      | Mean ± SEM                        | Mean ± SEM                        | Mean ± SEM            | S vs. F = n.s.†                   |
| sPD-L1 (pg/ml)       | 48.47 ± 1.48         | 48.72 ± 1.92                      | 48.16 ± 2.36                      | 51.89 ± 2.20          | F vs. HC = 0.007                 |
|                      |                      | Mean ± SEM                        | Mean ± SEM                        | Mean ± SEM            | A vs. HC = n.s.                   |
| sPD-L2 (ng/ml)       | 14.21 ± 0.36         | 13.16 ± 0.41                      | 15.47 ± 0.55                      | 11.76 ± 0.28          | S vs. F = 0.001                  |

* SEM = standard error of the mean. † n.s. = not significant.
forms. The soluble forms are usually generated by proteolytic cleavage of the membrane-bound forms (Zhu and Lang, 2017). Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) are known to induce the secretion of sPD-1 by T lymphocytes (Bommarito et al., 2017). These cytokines, TNF-α, IL-6, and IL-1β, are increased in the sera of patients with ALS and therefore may increase levels of sPD-1 in these patients (Hu et al., 2017). Furthermore, regulatory T (Tregs) lymphocytes are dysfunctional in patients with ALS, inhibiting immunosuppression, and thus augmenting the pro-inflammatory effector T (Teffs) lymphocytes responses and the possible cleavage of membrane-bound PD-1. The resultant increase in serum sPD-1 levels could further exacerbate the pro-inflammatory response (Henkel et al., 2013; Beers et al., 2017).

PD-1 inhibition has been proposed to mount an interferon-γ (IFN-γ)-dependent systemic immune response, leading to the recruitment of peripheral myeloid cells to the brain, and neuropathological and functional improvements in mice with Alzheimer’s-like disease (Baruch et al., 2016). On the other hand, another study showed that immunotherapy against PD-1 by itself is not sufficient to reduce amyloid pathology in APP/PS1 mice (Latta-Mahieu 2017). A more recent study found that although PD-1 was increased in the brains of mice with prion disease, the genetic deletion of PD-1 did not cause myeloid cell infiltration into the brain (Obst et al., 2018). In addition, genetic ablation of PD-1 did not lead to exacerbated pro-inflammatory responses which might be secondary to subsequent lack of serum sPD-1. Inhibiting the suppressive functions of PD-1 and exacerbating the pro-inflammatory responses in ALS by the increased production and secretion of IFN-γ would be counter-productive. In a mouse model of ALS-like disease, IFN-γ transcripts were increased in cervical as-well-as lumbar spinal cords of these animals (Beers et al., 2011a). Furthermore, elevated levels of IFN-γ have been demonstrated in cerebrospinal fluid and serum of patients with ALS (Liu et al., 2015). More importantly, several other studies have demonstrated that curtailting the pro-inflammatory responses in animal models of ALS slows disease progression and prolongs survival. Thus, in contrast to exacerbating pro-inflammatory responses in patients with ALS, suppressing these responses offers promising therapies for these patients (Beers et al., 2011b; Thonhoff et al., 2018).

The presence of increased levels of the metalloproteinases (MMPs) MMP-9 and MMP-13 has been described in inflammatory arthritis, and sPD-L1 and sPD-L2 can be regulated through proteolytic cleavage by MMPs (Bommarito et al., 2017). Two separate groups have found increases in both pro-MMP-9 and active MMP-9 in sera of patients with ALS relative to HC (Brkic et al., 2015). Furthermore, Fang et al. (2009) found increased levels of MMP-9 in cerebrospinal fluid from patients suffering from rapidly progressing ALS. The authors speculated that increased MMP-9 levels were associated with disease progression and disease burden. Elevated MMP-9 levels were also demonstrated in the spinal cords of mice with an ALS-like disease (Fang et al., 2010). Thus, elevated serum MMPs conceivably may be responsible for elevated levels of sPD-1 and sPD-L2 in patients with ALS.

The in vitro and in vivo pro-inflammatory properties of sPD-1 have been well documented (Guo et al., 2018). sPD-1 has been reported to enhance T-cell responses by interfering with the PD-1/PD-L1 pathway in autoimmune, tumor and viral infection systems. sPD-1 aggravates progression of symptoms in a murine model of collagen-induced arthritis, and increased serum sPD-1 has been reported in patients with
rheumatoid arthritis (Guo et al., 2018). The current report found that sPD-1 was elevated in the sera of patients with ALS compared with HC and positively correlated with disease burden; the greater the disease burden, the greater the serum sPD-1 levels. This increase in sPD-1 expression inhibits the PD-1/PD-L1 signaling pathway in T lymphocytes through negative feedback by blocking the membrane PD-1 binding site, which then reduces the inhibition of T lymphocyte activation and increases the activity of the pro-inflammatory response (Li et al., 2016). Furthermore, ex-vivo expansion of healthy control Tregs, lymphocytes known to suppress activated pro-inflammatory Teffs and macrophages, and found to be dysfunctional in patients with ALS, express increased levels PD-1 transcripts, but not increased levels of PD-L1 and PD-L2, compared with non-ex-vivo expanded Tregs (Beers et al., 2017; Faridar et al., 2020). Increased levels of PD-1 suggest these ex-vivo expanded Tregs are immunosuppressive and are currently in a Phase IIa clinical trial to determine if they reduce the pro-inflammatory responses and slow disease progression in patients with ALS (ClinicalTrials.gov NCT04055623; Thonhoff et al., 2018).

The pro-inflammatory role of sPD-L2 has not been as well characterized as that of sPD-1. However, one study showed that serum levels of sPD-1 and sPD-L2 were elevated in patients with systemic sclerosis (SSc) suggesting that sPD-1 and sPD-L2 contribute to disease development via the regulation of interactions with T and B lymphocytes (Fukasawa et al., 2017). The authors speculated that sPD-1 and sPD-L2 promote lymphocyte responses through blockade of the PD-1/PD-L2 pathway and that elevated levels of these proteins might result in lymphocyte activation. The elevated serum levels of sPD-L2 in patients with ALS suggest an enhanced peripheral pro-inflammatory response in these patients. Interestingly, there was also a positive correlation between serum sPD-1 and sPD-L2, as the serum levels of sPD-1 increased, so did the levels of serum sPD-L2.

The lack of increased sPD-L1 may be resolved by understanding the expression levels of PD-L1/PD-L2 on cells of the immune system. PD-L1 is ubiquitously expressed on the surface of many hematopoietic cells as well-as non-hematopoietic healthy tissues (Sun et al., 2018). However, PD-L2 is expressed on a much more limited population of cells that is restricted to macrophages and dendritic cells (DCs), and has a higher affinity for PD-1 than PD-L1. Zhao et al. (2017) recently demonstrated that macrophages/macrophages obtained from patients with ALS are activated and skewed towards a pro-inflammatory state in the peripheral circulation and correlated with rapid disease progression. Furthermore, an earlier study demonstrated immunohistochemically the presence of activated mature DCs in the ventral horns and corticospinal tracts of autopsy tissue harvested from patients with ALS (Henkel et al., 2004). Thus, activated monocytes/macrophages and DCs are likely to have PD-L2 cleaved from their cell surfaces, possibly by MMPs, contributing to the elevated serum levels of PD-L2 in patients with ALS.

As previously mentioned, ALS and tumor pathobiologies may be thought of as opposite ends of a detrimental versus beneficial spectrum of pro-inflammatory immune responses. The increased pro-inflammatory responses in patients with ALS leads to the possibility that there is less overall cancers in this population of patients. In a recent population database study, the overall risk of cancer at any site, as well-as the risk of lung cancer, was found to be reduced in patients with ALS (Gibson et al., 2016). The authors concluded that ALS, like other neurodegenerative diseases, may be protective against cancer. However, they also concluded that the pathogenic factors for this observation were not understood. The current study, and other studies demonstrating central and peripheral pro-inflammatory responses, as well-as dysfunctional Tregs, in patients with ALS suggest that these pro-inflammatory responses may be contributing to a lower risk of cancer in these patients (Beers et al., 2011b, 2017, 2020; Henkel et al., 2013; Zhao et al., 2017; Thonhoff et al., 2018). The increased serum levels of sPD-1 and sPD-L2 may not be independent of the generalized chronic low-level inflammatory responses in ALS. The specific contribution of sPD-1 and sPD-L2 to the overall inflammatory pathobiology in patients is not known. Nevertheless, the positive correlation of sPD-1 and sPD-L2 with serum levels of LBP in the same patients suggests that the pathobiology of sPD-1 and sPD-L2 in the inflammatory processes in patients with ALS may well be connected (Beers et al., 2020).

Patients with ALS have chronic and persistent low-grade systemic inflammation that is associated with a worse disease prognosis (Keizman et al., 2009). In contrast to ALS, cancer biology studies have shown that tumors escape immune surveillance by activating PD-1/PD-L2 pathways. However, the results of this study demonstrating elevated levels of serum sPD-1 and sPD-L2, and their correlations with disease burdens, provide evidence that the inhibition of the PD-1/PD-L2 pathways in T lymphocytes could contribute to the systemic pro-inflammatory response in ALS. Thus, enhancing the activation of the PD-1/PD-L2 pathways or attenuating their blockade by sPD-1 and sPD-L2 could be therapeutic strategies to decrease the systemic inflammation in patients with ALS.

Author contributions

DRB and SHA contributed to the study concept and design. DRB contributed to the acquisition, analysis and interpretation of data, and drafting of the manuscript. SW and JW performed the experiments. WZ, JRT, ADT, and AF provided critical reviews and revisions of the manuscript. All authors reviewed and contributed comments that were incorporated into the final version of this manuscript. Study supervision was provided by SHA.

Declaration of competing interest

DRB and SHA declare a conflict of interest with Implicit Bioscience. The remaining authors have declared no conflicts of interest.

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References

Baruch, K., Deczewska, A., Rozenzweig, N., Tsitoura-Kampoli, A., Sharif, A.M., Matcovitch-Natan, O., Ketters, A., David, E., Amit, I., Schwartz, M., 2016. PD-1 immune checkpoint blockade reduces pathology and improves memory in mouse models of Alzheimer’s disease. Nat. Med. 22 (2), 135–137.

Beers, D.R., Zhao, W., Liao, B., Kano, O., Wang, J., Huang, A., Appel, S.H., Jenny, S., Henkel, J.S., 2011a. Neuroinflammation modulates distinct regional and temporal clinical responses in ALS mice. Brain Behav. Immun 25 (5), 1025–1035.

Beers, D.R., Henkel, J.S., Zhao, W., Wang, J., Huang, A., Wen, S., Liao, B., Appel, S.H., 2011b. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. Brain 134 (5), 1293–1314.

Beers, D.R., Zhao, W., Wang, J., Zhang, X., Wen, S., Neal, D., Thonhoff, J.R., Alulimian, A.S., Shpill, E.J., Rezvani, K., Appel, S.H., 2017. ALS patients’ regulatory T lymphocytes are dysfunctional, and correlate with disease progression rate and severity. JCI Insight 2 (5), e89530.

Beers, D.R., Appel, S.H., 2019. Immune dysregulation in amyotrophic lateral sclerosis: mechanisms and emerging therapies. Lancet Neurol. 18 (2), 211–220.

Beers, D.R., Zhao, W., Neal, D.W., Thonhoff, J.R., Thome, A.D., Faridar, A., Wen, W., Wang, W., Appel, S.H., 2020. Elevated acute phase proteins reflect peripheral inflammation and disease severity in patients with amyotrophic lateral sclerosis. Sci. Rep. 10, 15295.

Bommarito, D., Hall, C., Taams, L.S., Corrigall, V.M., 2017. Inflammatory cytokines compromise programmed cell death-1 (PD-1)-mediated T cell suppression in inflammatory arthritis through up-regulation of soluble PD-1. Clin. Exp. Immunol. 188 (3), 455–466.

Bricke, M., Baluss, S., Libert, C., Vandenbroucke, R.E., 2015. Friends or Foes: matrix metalloproteinases and their multifaceted roles in neurodegenerative diseases. Mediat. Inflamm. 620581.

Brown, R.H., Al-Chalabi, A., 2017. Amyotrophic lateral sclerosis. N. Engl. J. Med. 377 (2), 162–172.

Fang, L., Huber-Abel, F., Teuchert, M., Hendrich, C., Dorst, K., Schattauer, D., Zettineisiel, H., Wlaschek, M., Schurrfeiter-Kochanek, K., Tumani, H., Ludolph, A.C., Bretschi-Meier, J., 2009. Linking neuron and skin: matrix metalloproteinases in amyotrophic lateral sclerosis (ALS). J. Neurosci. 285 (1-2), 62–66.

Fang, L., Teuchert, M., Huber-Abel, F., Schattauer, D., Hendrich, C., Dorst, K., Zettineisiel, H., Wlaschek, M., Schurrfeiter-Kochanek, K., Kapfer, T., Tumani, H.,
Ludolph, A.C., Brettschneider, J., 2010. MMP-2 and MMP-9 are elevated in spinal cord and skin in a mouse model of ALS. J. Neurol. 257 (1-2), 51–56.

Farid, A., Thome, A.D., Zhao, W., Thonhoff, J.R., Beers, D.R., Farenb., B., Masdeu, J.C., Appel, S.H., 2020. Restoring regulatory T-cell dysfunction in Alzheimer’s disease through ex vivo expansion. Brain Commun 2 (6), eca112.

Fukasawa, T., Yoshizaki, A., Ehatu, S., Nakamura, K., Salguna, R., Miura, S., Yamashita, T., Hirabayashi, M., Ichimura, Y., Taniguchi, T., Asano, Y., Shimizu, H., Kazoe, Y., Mawatari, K., Kitamori, T., Sato, S., 2017. Contribution of soluble forms of programmed death 1 and programmed death ligand 2 to disease severity and progression in systemic sclerosis. Arthritis Rheum. 69 (9), 1879–1890.

Gibson, S.B., Abbott, D., Farnham, J.M., Thai, K.K., McLean, H., Figueroa, K.P., Bromberg, M.B., Pulst, S.M., Lisa Cannon-Albright, L., 2016. Population-based risks for cancer in patients with ALS. Neurology 87 (3), 289–294.

Gu, D., Ao, X., Yang, Y., Chen, Z., Yu, X., 2018. Soluble immune checkpoints in cancer: production, function and biological significance. J. Immunotherapy Cancer 6 (1), 132.

Guo, Y., Walsh, A.M., Canavan, M., Wechalekar, M.D., Cole, S., Xuefeng Yin, X., Scott, B., Loza, M., Orr, C., McGarry, T., Bombardieri, M., Humby, F., Proudman, S.M., Pittalis, C., Smith, M.D., Friedman, J.R., Anderson, L., Madakamulli, L., Vie, D.L., Fearon, U., Nagpal, S., 2018. Immune checkpoint inhibitor PD-1 pathway is down-regulated in synovium at various stages of rheumatoid arthritis disease progression. PLoS One 13 (2), e0192704.

Hastabosn, M.P., Staff, N.P., Bornschlegl, S., Butler, G.W., Max, M.L., Kazamel, M., Zubair, A., Gastineau, D.A., Windebank, A.J., Dietz, A.B., 2017. Comprehensive immune profiling reveals substantial immune system alterations in a subset of patients with amyotrophic lateral sclerosis. PLoS One 12 (7), e0182002.

Haverkamp, I.J., Appel, V., Appel, S.H., 1995. Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. Brain 118 (3), 707–719.

Henkel, J.S., Engelhardt, J.I., Siklo, 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 (2), 221–235.

Henkel, J.S., Beers, D.R., Wen, S., Rivera, A.L., Toennis, K.M., Appel, J.E., Zhao, W., Moore, D.H., Powell, S.Z., Appel, S.H., 2013. Regulation T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. EMBO Mol. Med. 5 (1), 64–79.

Hu, Y., Cao, C., Qin, X.-Y., Yu, Y., Yuan, J., Zhao, Y., Yong Cheng, Y., 2017. Increased peripheral blood inflammatory cytokine levels in amyotrophic lateral sclerosis: a meta-analysis study. Sci. Rep. 7 (1), 9094.

Keizman, D., Rogowski, O., Berliner, S., Ish-Shalom, M., Maimon, N., Nefussy, B., Artamonov, I., Drory, V.E., 2009. Low-grade systemic inflammation in patients with amyotrophic lateral sclerosis. Acta Neurol. Scand. 119 (6), 383–389.

Latta-Mahieu, M., Elmer, B., Bretteville, A., Wang, Y., Lopez-Grancha, M., Gonist, P., Moindrot, N., Ferrari, P., Blanc, V., Schussler, N., Braud, E., Roudiere, V., Blanchard, V., Yang, Z.-F., Barnesoud, P., Bertrand, P., Roucourt, B., Carmans, S., Botzelbergs, A., Mertens, L., Winstmolders, C., Larsen, P., Hersley, C., McGady, T., Racke, M.M., Liu, L., Lu, J., O’Neill, M.J., Riddell, D.R., Ehneath, A., Nabel, G.J., Prudier, L., 2018. Systemic immune-checkpoint blockade with anti-PD1 antibodies does not alter cerebral amyloid-β burden in several amyloid transgenic mouse models. Glia 66 (3), 492–504.

Li, Y., Xiao, Y., Su, M., Zhang, R., Ding, J., Hao, X., Ma, Y., 2016. Role of soluble programmed death-1 (sPD-1) and sPD-ligand 1 in patients with cystic echinococcosis. Exp. Ther. Med. 11 (1), 251–256.

Liu, J., Gao, L., Zhang, D., 2015. Elevated levels of IFN-γ in CSF and serum of patients with amyotrophic lateral sclerosis. PLoS One 10 (9), e0136937.

Murdock, B.J., Zhou, T., Kashlan, S.R., Little, R.J., Goutman, S.A., Feldman, E.L., 2017. Correlation of peripheral immunity with rapid amyotrophic lateral sclerosis progression. JAMA Neurol 74 (12), 1446–1454.

Obst, J., Mancuso, R., Simon, E., Gomez-Nicola, D., 2018. PD-1 deficiency is not sufficient to induce myeloid mobilization to the brain or alter the inflammatory profile during chronic neurodegeneration. Brain Behav. Immun. 73, 708–716.

Sheean, R.K., McKay, F.C., Cretney, E., Bye, C.R., Perera, N.D., Tomas, D., Weston, R.A., Scheller, K.J., Dijouma, E., Menon, P., Schibeci, S.D., Marmash, N., Yerbury, J.J., Nutt, S.L., Booth, D.R., Stewart, G.J., Kieman, M.C., Vucic, S., Turner, B.J., 2018. Association of regulatory T-cell expansion with progression of amyotrophic lateral sclerosis: a Study of Humans and a Transgenic Mouse Model. JAMA Neurol 75 (6), 681–689.

Sun, C., Mezzadra, R., Schwamacher, T.N., 2018. Regulation and function of the PD-L1 checkpoint. Exp. Ther. Med. 11 (3), 695–709.

Traini, C.M., Halsey, W.S., Hughes, A.M., Sathe, G.M., Livi, G.P., Fan, G.-H., Traini, C., Brosnan, M.P., 1998. B-cell depletion to induce myeloid mobilization to the brain or alter the in vivo inflammatory profile during chronic neurodegeneration. Brain Behav. Immun. 73, 708–716.

Thonhoff, J.R., Beers, D.R., Zhao, W., Pleitez, M., Simpson, E.P., Berry, J.D., Cudkowicz, M.E., Appel, S.H., 2018. Expanded autologous regulatory T-lymphocyte infusions in ALS: a phase I, first-in-human study. Neurol. Neuroimmunol. Neuroinflamm. 5 (4), e452.

Zhao, W., Beers, D.R., Hooten, K.G., Sieglaff, D.H., Zhang, A., Kalyana-Sundaram, S., Appel, S.H., 2017. Characterization of gene expression phenotype in amyotrophic lateral sclerosis: a Study of Humans and a Transgenic Mouse Model. JAMA Neurol 75 (6), 681–689.