CD45 expression in peripheral T cells of ALS patients during disease progression

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Introduction

Amyotrophic Lateral Sclerosis (ALS) is a relentlessly progressive disease of motor neurons of unknown aetiology and lethal course, leading to paralysis and death generally in 3-5 years from onset (Boillee et al, 2006). Although much has been made to understand ALS pathogenesis, the diagnosis is presently made on a clinical basis when neuronal death is already widespread. Effective therapy is available for ALS: the only approved compound, riluzole (acting as an anti-glutamatergic), slightly prolongs survival, but has no effect either in relieving existing symptoms, or in partially recovering motor functions (Miller et al, 2007). The development of new drugs is difficult because of the lack of molecular indicators of therapeutic efficacy, useful for patient follow up in clinical trials. In this regard, peripheral alterations associated to ALS, regarding degeneration and death of VACHT+ and cells in blood, could be valuable. Systemic deregulation of immune molecules was reported in both ALS mouse and the SOD1-G93A mouse (Zang et al, 2005; Erie et al, 2007; Banerjee et al, 2008). The dysfunction observed applies to innate and adaptive immune systems and seems associated to inflammatory phenomena (McGeer and McGeer, 2002; Graves et al, 2004; Holmoy, 2008). In particular, T lymphocytes were shown activated in ALS patients, and the number of the activated lymphocytes was found decreasing during ALS progression (Zang et al, 2005). In contrast, CD45+ T lymphocyte subpopulations appeared altered in sporadic ALS patients in comparison with caregivers (Banerjee et al, 2008). Importantly, similar observations were made on other neurodegenerative conditions distinct from ALS, such as in Alzheimer’s disease (AD) (Tan et al, 2004) and Parkinson’s disease (PD) (Bas et al, 2002; Baba et al, 2005). Such reports prompted a number of research groups to investigate to involvement of systemic inflammatory pathways in ALS etiopathogenesis and the correlation between systemic inflammation and immune activation, i.e., regarding systemic features of oxidative stress (Simons et al, 2004) or of activation of the Tumor Necrosis Factor (TNF) proinflammatory pathway (Poloni et al, 2000; Cereda et al, 2008) on this ground is based the rationale of the work herein presented. However, while most recent studies mainly focused on CD45+ T lymphocytes, we included in our analysis all peripheral blood cell markers, including T and B lymphocytes and macrophages.

Methods

Seventy-eight patients, diagnosed with ALS based upon EIC criteria, were enrolled in the study at the Neurorehabilitation Unit of Azienda Ospedaliera-Universitaria Pisana upon approval by the Ethical Committee. Patients had not history of familial ALS and are described in detail in the Supplementary Table. Patients had signed informed consent prior to the study. At each scheduled time point the presence of inflammatory diseases was ruled out by white blood cell counts and red blood cell sedimentation rate. Peripheral blood samples were drawn for serum and plasma preparation. For plasma preparation, samples were collected in Vacutest-Kima tubes with K3EDTA 1.8 mg/ml. For the fluorocytometric characterization, 100 microliters of plasma were incubated 15’ at room temperature with combinations of 6 distinct fluorochromes, namely FITC, PE, PerPC, PE-Cy7, APC, APC-Cy7. Antibodies against CD44, CD45Ra, and soluble factors, as listed in the methods. The subsequent step was performed in a flowcytometry device with a gain of 90, and the acquired signal was stored in a computer. The subsequent step was performed in a flowcytometry device with a gain of 90, and the acquired signal was stored in a computer. The statistical analysis was performed using SPSS version 7.0 (SPSS Inc, Chicago, Illinois, USA). A p-value <0.05 was considered statistically significant. Each statistic test is described in detail in the methods section.

Results

Figures 1-4

Regarding the expression of CD45 at the 3rd week from therapy starting, the average value of CD44+ CD45RA+ CD45R0+ lymphocytes, and the amount of CD25+ T lymphocytes (Pearson’s coefficient = 0.395, p value <0.0001) increases, and so does time from therapy starting, the average value of CD25% increases, as well as its spread around the average. It is worth noting that no significant changes in total peripheral lymphocytes is observed during the same window.

ALS is generally regarded as a complex syndrome, characterized by a certain degree of clinical and molecular heterogeneity when considering the overall patient population. Notably, it is believed that pharmacological research in this field has been rather scarcely successful because aimed at curing the totality of ALS population, instead of a well-characterized patient subgroup within a plethora of possibly distinct ALS phenotype-associated molecular defects. The heterogeneity of molecular defects and of response to therapy could also have caused the controversy and low statistical significance of recent drug trials: as far as we know, in recent clinical trials only a small minority of ALS patients might have been addressed to a given therapy, maybe over a much larger number representing the background. The results herein presented could therefore be quite valuable, since contributing up to build an efficient subclassification of such a heterogeneous class of patients. Overall, the most interesting conclusion of our report is that a decrease in the expression of CD45RA and CD45R0 on peripheral lymphocytes discriminates a peculiar subgroup of ALS patients within a heterogeneous superfamily of ALS patients. We have also observed that the sum of CD45RA+ CD45R0+ lymphocytes and low CD25+ lymphocytes at the start of riluzole therapy; interestingly, as time from the therapeutic start increases, both CD45 and CD20 values tend to return to the reference values, common to the healthy population and to the rest of the ALS population examined. Hence, for a given subgroup of ALS patients, an objective parameter might have emerged, which might account for the effects of riluzole therapy and could possibly be exploited in assessing the efficacies of novel drugs in clinical trials. As far as we know, this is the first time that a homogeneous group of ALS patients could be followed over riluzole therapy based on the normalization of a peculiar disease-related parameter.