HERV-K, Immune Response in ALS

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Contributor: Leonardo Sechi

Human endogenous retrovirus (HERV)-K env-su glycoprotein has been documented in amyotrophic lateral sclerosis (ALS), where HERV-K env-su 19–37 antibody levels significantly correlated with clinical measures of disease severity. Herein, we investigated further the humoral and cell-mediated immune response against specific antigenic peptides derived from HERV-K in ALS. HERV-K env glycoprotein expression on peripheral blood mononuclear cells (PBMCs) membrane and cytokines and chemokines after stimulation with HERV-K env 19–37 and HERV-K env 109–126 were quantified in patients and healthy controls (HCs). HERV-K env glycoprotein was more expressed in B cells and NK cells of ALS patients compared to HCs, whereas HERV-K env transcripts were similar in ALS and HCs. In ALS patients, specific stimulation with HERV-K env 109–126 peptide showed a higher expression of IL-6 by CD19/B cells. Both peptides, however, were able to induce a great production of IFN-γ by stimulation CD19/B cells, and yielded a higher expression of MIP-1α and a lower expression of MCP-1. HERV-K env 19–37 peptide induced a great production of TNF-α in CD8/T cells. In conclusion, we observed the ability of HERV-K to modulate the immune system, generating mediators mainly involved in proinflammatory response.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects both upper and lower motor neurons, determining progressive paralysis and death, within 3–5 years post diagnosis. About 5–10% of ALS cases are familial, the remaining are apparently sporadic of unknown etiology [1]. A poor knowledge of ALS pathophysiology is the major reason for the absence of specific diagnostic tests in clinical practice and the lack of an effective treatment. Thus, it is important to find reliable diagnostic and prognostic markers that can guide the clinical diagnostic process at an early stage of the disease. There is no cure for ALS, and the FDA-approved drugs are only marginally effective in slowing the progression of the disease by a few months [1]. Advances in understanding pathogenic mechanisms are essential to identify new possible therapeutic interventions. Several studies have demonstrated the presence of reverse transcriptase activity in serum samples of both ALS patients and their unaffected relatives [2][3], indicating that a common inherited endogenous retrovirus may be the trigger of the disease [4]. Endogenous retroviral sequences constitute 8% of human genomes, in which they have been integrated through repeated infections during evolution [5]. In particular, RNA sequences of the human endogenous retrovirus of the K family (HERV-K) have been detected in motor neurons of ALS patients [6][7], and it has been observed that increased expression of HERV-K envelope protein in upper and lower motor neurons was neurotoxic

and able to cause cellular degeneration [7], thus indicating the reactivation of HERV-K in the affected tissues. Moreover, in a transgenic mouse model, increased HERV-K envelope protein expression in motor neurons induces a clinical and pathological phenotype that resembles ALS, strongly suggesting that HERV-K may play a contributing role in the pathophysiology of this disorder [7].

Recently, we investigated the specific humoral immune response against four antigenic peptides derived from the HERV-K env-su glycoprotein in serum and cerebrospinal fluid (CSF) of ALS patients, reporting a significant immune response directed toward the HERV-K env-su $_{19–37}$ peptide both in serum and CSF of ALS patients, but not in healthy controls (HCs). In addition, we observed a specific intrathecal IgG synthesis against HERV-K peptides and a functionally intact blood–brain barrier in most of the patients analyzed, indicating that there is active antibody production within the CNS. Moreover, in ALS patients, the HERV-K env-su $_{19–37}$ antibody levels were significantly correlated with clinical measures of disease severity, both in serum and CSF [8]. In this paper, we shed light on the role of HERV-K in the pathophysiology of ALS by evaluating the humoral and cell-medi ated immune response to HERV-K in ALS patients compared to HCs, using different methodological approaches: (1) cytometric analysis to quantify HERV-K env-su glycoprotein expression on peripheral blood mononuclear cells (PBMCs) membrane and test the T and B mediated immune response after antigenic stimulation with HERV-K env $_{19–37}$ and HERV-K env $_{109–126}$, by quantification of Interleukin-6 (IL-6), Interferon- γ (IFN-γ), Tumor Necrosis Factor-α (TNF-α), Macrophage Inflammatory Protein-1α (MIP-1α), and Monocyte Chemoattractant Protein-1 (MCP-1); (2) investigation of HERV-K transcripts in PBMCs by RT qPCR method; (3) analysis by ELISA of specific antibodies against env-surface peptides; and (4) quantification of IgG1 and IgG4 subclasses by nephelometry. We detected IgG1 plasma levels significantly higher in ALS patients compared to HCs; conversely, no difference between the two groups was observed for IgG4 plasma levels.

The results obtained highlighted the ability of HERV-K to modulate the immune system at different levels, generating mediators mainly involved in proinflammatory response. Of note, despite depletion, B cells of ALS patients express higher levels of HERV-K env protein compared to HCs feeding the inflammatory loop, by induction of a continuous proinflammatory stimulus of T lymphocytes sustained by exposition of viral protein on B lymphocytes membrane.

2. Discussion

ALS is characterized by heterogeneous clinical signs and symptoms, and a definite diagnosis may require 13 to 18 months, once of other disorders that can affect upper and lower motor neurons have been excluded [9][10].

The identification of specific and sensitive circulating biomarkers for ALS could permit an early diagnosis and avoid unnecessary and potentially harmful therapeutic trials. In particular, an increase in HERVs expression has been observed in some neurological diseases, including ALS, although there is no evidence that this may be the primary causative factor for the pathology.

In a previous study, we documented an HERV-K env antigen humoral immunity response by detecting specific IgG antibodies in serum and CSF of Sardinian patients affected by ALS, with respect to other neurological diseases [8]. In this study, to better secure the role of retroviruses in ALS, and validate the use of HERV-K as a possible diagnostic biomarker, we expanded the size of our previously studied cohort to 55, and respective healthy controls to 102, confirming the data obtained in the previous paper [8]. We also used different methodological approaches to better understand the role of HERV-K on humoral and cell-mediated immune responses both by using cytometric analysis to quantify the HERV-K env peptides expression on the PBMCs membrane of ALS patients and controls and by investigating HERV-K transcripts in PBMCs by RT-qPCR method. Notably, PBMCs have been proposed as a good non-invasive option for studying ALS [11].

The analysis of viral protein expression on lymphomonocytes showed that HERV-K env protein was expressed with greater percentage frequency in B cells of ALS patients. This finding supports the results of ELISA tests in serum and CSF showing that the production of specific immunoglobulins towards selective retroviral peptides was significantly higher in patients with ALS with respect to HCs [8]. Instead, the analysis of the HERV-K env transcripts has not proven any significant difference between patients and controls as previously observed by Garson [12]. Interestingly, other HERVs families have been associated to different neurological diseases, such as HERV-W to multiple sclerosis (MS) from different authors [13][14], and HERV-W env expression on the membrane of PBMCs in MS patients seems modulated by natalizumab [15]. Recently, we have also shown that circulating antibody levels directed against the HERV-W env-su $_{93–108}$ and HERV-W env-su $_{248–262}$ peptide fragments are different in different CNS demyelinating disorders [16][17].

Based on our findings, HERV-K env protein appeared to be poorly expressed on the CD4+ and CD8+ lymphocyte membranes of both ALS patients and controls (data not shown), despite retroviral antigens also determining a humoral response T helps mediate.

The specific immune response which T and B mediated after stimulation of PBMCs with two antigenic peptides derived from the HERV-K env-su glycoprotein, namely HERV-K env $_{19–37}$ and HERV-K env $_{109–126}$, in patients with ALS and HCs, showed significant differences among the cytokines produced, indicating a good and broad reactivity of the immune system. In particular, the expression of IL-6 by CD19/B cells was significantly different after stimulation with HERV-K env $_{109–126}$ peptide in ALS patients in comparison to HCs, suggesting that this peptide is likely responsible for the B-cell activation, considered the autocrine activity of this cytokine. Instead, the HERV-K env $_{19–37}$ peptide seems able to foster a pro-inflammatory response by stimulation of CD19/B cells and a statistically significant greater production of IFN-γ. In CD19/B cells, both peptides were not able to stimulate the TNF-α production, while the HERV-K env $_{19–37}$ peptide induced a greater production of TNF-α in CD8/T cells.

The mechanism for this latter effect is unknown. We believe it might be due to an insufficient immune innate response in ALS patients after a 6-hour stimulus of PBMCs. This is consistent with an increased retroviral expression observed on monocytes of HCs compared to patients, as TNF-α is a pro-inflammatory cytokine that appears at the early stages of the innate immune response. Previous studies have reported higher levels of IL-6 in ALS patients in comparison to HCs [18][19][20][21][22] and rising IL-6 levels in plasma were also associated with risk

for disease progression [18]. Interestingly, although IL-6 may have an established bivalent role in inflammation, namely both a pro and anti-inflammatory action, a peripheral IL-6 upregulation usually is related to an inflammatory cell response also able to induce an endothelial cells damage at blood–brain barrier. [22]. Thus, our data support the possibility that an increased expression of HERV-K can stimulate IL-6 and IFN-γ determining a chronic inflammatory state through a T lymphocytes/Th1-type cytokines response which, in patients genetically predisposed, may play a role in worsening the disease course.

Moreover, we studied the expression of MIP-1α and MCP-1 by CD19/B-cells stimulated with both peptides, and found a significant increase in the expression of MIP-1α in ALS patients. This finding fits previous data in the literature showing either a negative correlation between expression of MIP-1α and disease progression rate or a positive correlation with disease duration, thus suggesting a possible protective role of this chemokine on ALS outcome [23]. The antigenic specific stimulation generated by HERV-K env $_{19–37}$ and HERV-K env $_{109–126}$ contribute to the specific increase in MIP-1 α levels as observed in serum and CSF by other authors [19][23]. Regarding MCP-1, we document a lower expression by CD19/B cells in ALS patients compared to HCs, following stimulation with both peptides. As higher MCP-1 levels were associated with worse disease severity and faster progression by several authors [19], and due to the HERV-K related lower expression of this chemokine documented in our study, it is likely to work together with the increased expression of MIP-1α to produce a better ALS outcome.

In this context, in order to investigate which IgG subclass of antibodies anti-HERV-K were predominant in patients with ALS, we determined plasma levels of IgG1 and IgG4 immunoglobulins. This is an important issue in order to clarify if the high levels of IgG found in ALS are protective (IgG4) or harmful (IgG1) [24]. Indeed, recent data have indicated that IgG4 antibodies may fulfil a protective role dampening the more harmful effects of IgG1 when directed against the same epitopes [24]. In addition, we evaluated if the observed IgG1 and IgG4 production could be correlated with disease progression and prognosis. We detected IgG1 plasma levels significantly higher in ALS patients compared to HCs; conversely, no difference between the two groups was observed for IgG4 plasma levels. Of note, IgG1 levels were not significantly different in newly diagnosed ALS patients with respect to long-survivor patients, thus this humoral finding does not have prognostic significance.

Interestingly, in long-survivor ALS patients, IgG1 and IgG4 plasma levels were both significantly increased with respect to HCs. This data indicates that, in long-survivors, the detrimental effect of increased IgG1 levels in plasma may be progressively dampened by the parallel increment in plasma of IgG4 levels. The mechanisms behind the pathological contribution of HERV-K activation in ALS remain yet to be completely clarified. Here, we document divergent mechanisms, related to humoral and cell-mediated immune response to antigenic peptides derived from HERV-K in ALS patients. In this context, our study provides useful information to better understand the possible role of endogenous retroviruses in neurodegenerative diseases. In particular, we highlighted the ability of HERV-K to modulate the immune system at different levels, generating mediators mainly involved in proinflammatory response. Of note, despite depletion, B cells of ALS patients express higher levels of HERV-K env protein compared to HCs feeding the inflammatory loop, by induction of a continuous proinflammatory stimulus of T lymphocytes sustained by exposition of viral protein on B lymphocytes membrane. Furthermore, we better understand the role of the cytokine IL 6 in the early stage of ALS as responsible for the injury of the BBB, such that

to support neuronal damage. Certainly, an early knowledge is sorely needed to diagnose ALS, as recently described by Keon et al. [25]. Adequate translation of our results into clinical practice may speed up the use of HERV-K as biomarker of disease progression due to its ability to modulate cytokines and chemokines as mediators of inflammation partly responsible for motor neurons damage.

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