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In-situ expression of Interleukin-18 and associated mediators in the human brain of sALS patients: Hypothesis for a role for immune-inflammatory mechanisms

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ABSTRACT

Recent studies reported over-expression of a cytokine (Interleukin (IL)-18) in the *serum* of sporadic amyotrophic lateral sclerosis (sALS) patients. Here, we report on the first-time detection of in-situ expression of activated IL-18 in the human brain in sALS patients. We also detected cerebral in-situ expression of key-molecules known to be closely related to the molecular network associated with the activation/secretion of IL-18 cytokine, namely, the receptor-interacting serine/threonine-protein kinase 3 (RIPK3 or RIP3), NOD-like receptor pyrin domain containing 3 (NLRP3)-inflammasome, and activated caspase-1.

These findings suggest and allow to hypothesize that there might be a role for this cytokine network in molecular mechanisms associated with or implicated in the physiopathology of this neurodegenerative disorder.

Introduction/background

Amyotrophic lateral sclerosis (ALS) is a devastating and rapidly progressing condition.

It is one of the severest neurodegenerative diseases that involves upper- and lower motor neurons, with typical limbs-, trunk-, and bulbar involvement, causing death usually from neurogenic muscle weakness. Many cases, besides, show or evolve with a distinctive component, namely fronto-temporal atrophy and/or dementia (FTD); a clinico-pathological spectrum of ALS/FTD has thus emerged [1,2], and insights into the wider implication of supra-spinal involvement of the CNS in this condition are expanding. Recently, imaging studies suggested involvement of basal ganglia [3]. Besides, the reported involvement of regions extending beyond the primary motor cortex (the post-central gyrus sensorimotor area, prefrontal cortex, and the anterior part of cingulated gyrus) raised the concept of ALS being a multisystem disorder with extramotor involvement [4].

Only very few cases are familial, mostly with autosomal-dominant inheritance. Nearly 95% are reportedly sporadic (sALS) [5], with no known aetio-pathogenic mechanism.

Studies revealed that most adult-onset neurodegenerative diseases display a somewhat sustained activation of elements of the immune-inflammatory system in the CNS of affected patients [6,7]; such hyperactivity of the innate immune response in neurodegenerative disorders [8] seemingly participates at least in the amplification of the disease in those conditions [9,10].

In ALS in particular, immune-inflammatory mechanisms were suggested to amplify motor-neuronal degeneration in the spinal cord [11]. Cytokines (which are key immune-inflammatory mediators) have been reported in several neurodegenerative conditions, and activated neural cells can produce cytokines [12].

A recent study reported upregulation of IL-18 cytokine in the *sera* of sALS patients [13]. To-date, however, we found no human brain studies involving immuno-histochemical (IHC) cytokine explorations in sALS.

The hypothesis

Growing evidence on the implication of proinflammatory cytokines in cerebral neuronal dysfunction and cell death in several neurological disorders (such as multiple sclerosis, Alzheimer's dementia, Parkinson, and others...), together with the recent

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reporting of overexpression of IL-18 (a known inflammatory cytokine) in the *sera* of patients with sALS [13], prompted us to hypothesize that this cytokine (IL-18) could take part in physiopathological mechanisms underlying cerebral neuronal injury/-damage in sALS.

Evaluation of the hypothesis

In order to find out if IL-18 could possibly be implicated in neural cell injury/damage particularly in the brain, it was imperative to verify and explore whether this cytokine (IL-18) and eventually related molecules, were actually present and expressed within the brain itself.

Empirical data/supportive results

To that aim, we carried-out a *retrospective neuropathological* study to explore sALS brains for eventual expression of IL-18 cytokine in cerebral tissue, and to see if related immune-inflammatory mediators/network were activated in the CNS of sALS patients.

We explored archived paraffin-embedded tissue-blocks from four human brains for the eventual presence of the IL-18 cytokine (in brain tissues), and we also looked for key-mediators closely related to IL-18, namely RIP3, caspase-1 and the NLRP3-inflammasome which are all closely implicated in the production of the active form of the cytokine IL-18.

All specimens were from post-mortem brains of patients with known sporadic ALS (sALS), autopsied in our Belgian university centers.

All autopsies were conducted in accordance with ethical and legal rules applied in our institutions. The study was approved by the Ethical Committee of our university center (CHU BRUGMANN; Ref: CE 2014/163).

Tissue blocks included in this neuro-molecular exploration involved cerebral regions reportedly mostly impacted on clinical grounds (fronto-temporo-parietal cortex). Besides, we included deep gray nuclear structures, namely, striatum, for comparison. We also included tissue blocks from another (fifth) brain from a patient with no known primary CNS pathology, to serve as "external" control.

All formalin-fixed, paraffin-embedded blocks (from the 5 brains) were serially cut and histological sections were first studied with routine stains and then processed for in-situ immuno-histochemical (IHC) detection of the various molecules searched for in this study.

In-situ IHC explorations on tissue-sections from all cases were conducted in identical laboratory conditions. IHC staining was performed on 5μ-thick sections from all brains. Immune-labeling techniques and, all procedural details for the in-situ detection of each of the molecules implicated in this study basically relied on techniques we described in previous studies [14–16]. In the present exploration, we used the following antibodies and concentrations: IL-18 (1:75, ab137664, Abcam, ON, Canada), RIP-3 (1:25, ab180535, Abcam, ON, Canada), NLRP-3 (1:100, sc-66846, Santa Cruz Biotechnology, TX, USA), and activated caspase-1 (1:25, sc-22164, Santa Cruz Biotechnology TX, USA).

In-situ immuno-histochemical (IHC) labeling showed strong cytokine immune-reactivity for IL-18 in cortical neurons of sALS brains explored in this study (Fig. 1A and B). We also found some

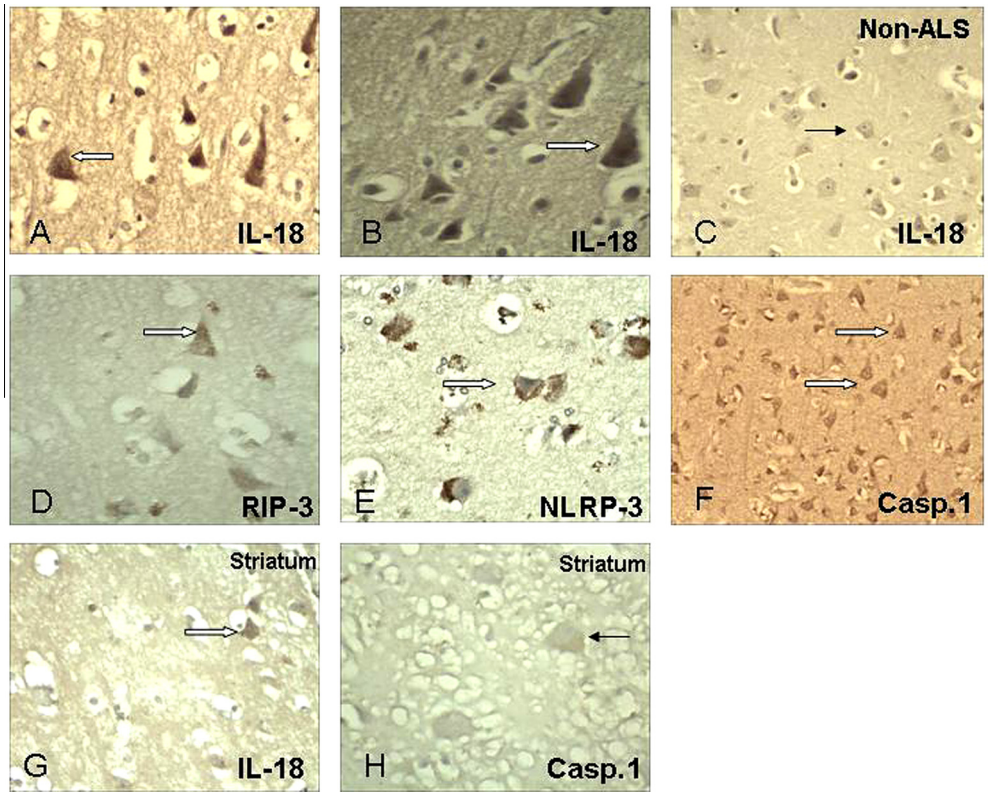

Fig. 1. In-situ immuno-histochemical labeling showing expression of IL-18 and related immune-inflammatory mediators in the human brain of sALS patients. A, B, and C, compare IL-18 expression in the cerebral cortex from the first and second sALS patients (A and B respectively) and a non-ALS case (C); note strong immune-labeling in pyramidal neurons of sALS brains (white arrows). Neural cells in C (dark arrow) practically show no immune-staining. D, E, and F, (from case #1; the same brain as A), show cerebro-cortical immune expression for immune-inflammatory mediators implicated in the production of IL-18, namely, RIP3 (D), NLRP-3 (E), and Caspase-1 (F). G and H show the pattern of immune-expression of IL-18 (G) and Caspase-1 (H) in the striatum (G comes from the 3rd ALS case, and, H from case #2; the same brain as in B). White arrows show labeled neural cells, whereas dark arrows point to non-labeled/weakly-labeled cells (C and H). Original magnifications: ×400 (B, D, G, and H); ×320 (A and E); ×200 (C); ×160 (F).