Circulating interleukin-15 and RANTES chemokine in Parkinson’s disease


Interleukin-15 promotes T-cell proliferation, induction of cytolitic effector cells including natural killer (NK) and cytotoxic cells and stimulates B-cell to proliferate and secrete immunoglobulins. RANTES is a C-C beta chemokine with strong chemoattractant activity for T lymphocytes and monocytes. Objectives – The objective of our study was to find out whether IL-15 and RANTES are involved in the possible inflammatory reactions of PD. Patients and methods – We measured by immunoassay serum IL-15 and RANTES levels in 41 patients with PD in comparison with serum levels in 19 healthy subjects age and sex-matched. IL-15 and RANTES levels were correlated with sex, age, disease duration, H-Y stage and the UPDRS III score in all the studied groups and were also correlated with treatment status in PD patients. Results – The PD group presented with significantly increased RANTES levels as compared to the control group ($P = 0.0009$). No difference was observed as regards IL-15 levels. A strong and significant correlation between RANTES levels and UPDRS III score was observed in PD patients ($R_s = 0.42$, $P = 0.007$). Untreated patients had significantly higher RANTES levels as compared to the controls. Conclusions – Our findings may suggest a recruitment of activated monocytes, macrophages and T lymphocytes to sites of inflammation in the central nervous system of PD patients.

Introduction

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by dopamine depletion in the nigrostriatal system. Mitochondrial dysfunction, oxidative stress, environmental toxins, and deficient neurotrophic support have been implicated in the etiopathogenesis of cellular degeneration and death in PD (1–3). However, the exact mechanisms leading to this degeneration are unknown. Immune factors have been proposed as one of the possible mechanisms by which cellular death occurs. Indeed, class II major histocompatibility complex (MHC) HLA-DR-positive reactive microglia have been demonstrated in the substantia nigra of patients with PD (4). These activated microglia were also positive for tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are known to have a neuroprotective function. MHC class II-positive microglia are a sensitive index of neuropathological change and are actively associated with damaged neurons and neuritis (5). Also the level of β2-microglobulin, the light chain of MHC class I, was found to be increased in the striatum of PD patients compared with control subjects (6).

Elevated levels of interleukin-2 (IL-2) have been reported in the striatum, and elevated levels of inflammatory cytokines, such as IL-1β, IL-2, IL-6, epidermal growth factor, and transforming growth factor (TGF)-α have been reported in the striatal dopaminergic neurons (7, 8). IL-15 is a pro-inflammatory cytokine produced by activated blood monocytes, macrophages, dendritic cells...
and activated glial cells (9–11). It promotes T-cell proliferation, induction of cytolytic effector cells including natural killer (NK) and cytotoxic cells and stimulates B cells to proliferate and secrete immunoglobulins (12, 13). It also induces the expression of chemokines, such as regulated upon activation of normal T cell expressed and secreted (RANTES) and macrophage inflammatory protein (MIP)-1α.

It has been demonstrated that activated microglia can produce both α- and β-chemokines, potent activators and chemoattractants for leukocyte and monocytes/macrophages, such as keratinocyte chemoattractant, MIP-1α, MIP-1β, MIP-2, macrophage chemoattractant protein (MCP)-1 and RANTES in response to experimental stimulation by lipopolysaccharides (14–17). Chemokine receptors such as CXCR2, CXCR3, CXCR4, CCR3, and CCR5 have also been reported for microglia in vitro and/or in vivo (18, 19). Chemokines are released by activated macrophages, microglia, astrocytes, and inflammatory cells acting like mediators of inflammation with selective chemoattractant properties. They regulate recruitment and migration of cells to sites of inflammation (20, 21). RANTES is a C-C beta-chemokine with strong chemoattractant activity for T lymphocytes and monocytes (22). To find out whether IL-15 and RANTES are involved in the possible inflammatory reactions of PD, we measured IL-15 and RANTES serum levels in patients with PD compared with healthy subjects. We divided our PD patients into three subgroups [treated with l-dopa, treated with agonist alone (pramipexole) or agonist plus l-dopa, and untreated] to investigate the effect of anti-parkinsonian treatment in the possible immune disturbances acting as part of pathogenetic mechanisms of the disease.

Patients and methods

After obtaining informed consent, 60 subjects who enrolled in the study were divided into two groups. (i) The PD group comprised 41 patients with clinically definite PD, diagnosed according to the criteria of Larsen et al. (23). (ii) The control (CTRL) group comprised 19 healthy age- and sex-matched individuals. Twenty of the PD patients were untreated, nine were receiving l-dopa only, and 12 were receiving dopamine agonist treatment as monotherapy or combined with l-dopa.

Patients with evidence of systemic inflammation on clinical examination or serum biochemical tests (increased number of white blood cells, elevated C-reactive protein, elevated erythrocyte sedimentation rate) were excluded. Patients who were using non-steroidal anti-inflammatory drugs, acetylsalicylic acid, steroids, or statins during the last 2 months before enrollment were also excluded from the study.

Sample collection, IL-15, and RANTES assay

Non-fasting blood samples were obtained from a peripheral vein and centrifuged within 1 h. Serum concentrations of circulating IL-15 were measured by immunoassay using commercially available enzyme-like immunosorbent assay (ELISA) kits (R and D systems, Minneapolis, MN, USA). Serum samples diluted 1 : 3 were added in duplicate to microtiter wells and assayed according to routine procedures. Optical densities were determined by means of a micro-ELISA reader (LP 400; Pasteur Diagnostics, France). Values were calculated by comparison with a standard curve that was generated with IL-15 standards. The limit of detection was 2 pg/ml.

Serum concentrations of RANTES were measured by using commercially available ELISA kits (R and D systems). Undiluted serum samples were added in duplicate to microtiter wells and assayed according to the manufacturer’s protocol. Optical densities were determined by means of a micro-ELISA reader (LP 400; Pasteur diagnostics). Values were calculated by comparison with a standard curve generated with serum RANTES standards. The limit of detection of serum RANTES assay was 0.002 ng/ml.

Statistical analysis

All variables were checked for normality and homogeneity of variances by the Shapiro-Wilk’s and Levene’s tests, respectively. Because of deviations from the normal distribution and/or heterogeneity of variances, nonparametric tests (Mann–Whitney test, Kruskal–Wallis test followed by Dunn’s test for multiple comparisons) were used for comparison of IL-15 and RANTES levels between patients and controls, and data are presented as medians and quartiles. The Spearman rank correlation coefficient, t-test, one-way ANOVA, and chi-squared test were also used as appropriate.

Results

Results are summarized in Table 1 and Fig. 1. Patients and controls did not differ significantly between each other with respect to age and sex. The PD group presented with significantly increased
RANTES levels compared to the controls ($P = 0.0009$), but no difference was observed as regards IL-15 levels. Sex, age, disease duration, and Hoehn-Yahr (H-Y) stage did not affect either IL-15 or RANTES levels. A strong and significant positive correlation between RANTES levels and Unified Parkinson Disease Rating Scale (UPDRS) III score was observed in PD patients ($R_S = 0.42$, $P = 0.007$, Fig. 2). Untreated PD patients had significantly higher RANTES levels compared to the control group (Dunn’s post hoc test, following Kruskal–Wallis test, $P = 0.01$). However, despite some numerical differences, the treated PD subgroups did not differ significantly compared to the controls (Table 2, Fig. 3).

**Discussion**

In this study we have provided evidence that serum RANTES levels are increased in the serum of PD patients ($P = 0.0009$), but no difference was observed as regards IL-15 levels. Sex, age, disease duration, and Hoehn-Yahr (H-Y) stage did not affect either IL-15 or RANTES levels. A strong and significant positive correlation between RANTES levels and Unified Parkinson Disease Rating Scale (UPDRS) III score was observed in PD patients ($R_S = 0.42$, $P = 0.007$, Fig. 2). Untreated PD patients had significantly higher RANTES levels compared to the control group (Dunn’s post hoc test, following Kruskal–Wallis test, $P = 0.01$). However, despite some numerical differences, the treated PD subgroups did not differ significantly compared to the controls (Table 2, Fig. 3).
patients with PD compared with CTRL subjects. The levels of RANTES and other chemokines are linked to the pathogenetic mechanisms of some central nervous system (CNS) disorders such as multiple sclerosis, Alzheimer’s disease, and brain ischemia (24–28). In two recent studies no associations were found between functional DNA polymorphisms at the genes encoding chemokines MCP-1, RANTES, and chemokine receptors 5 and 2 (CCR5 and CCR2), and the risk or age of onset of PD (29, 30). Our results suggest a probable recruitment of activated monocytes, macrophages and T lymphocytes to sites of inflammation in the CNS of PD patients and injury through interaction with RANTES receptor CCR5. The systemic presence of RANTES in our PD patients may reflect a central or peripheral source of activation. To clarify the issue of the source of circulating RANTES, its expression and production by activated blood mononuclear cells should be assessed.

Serum IL-15 levels were not different between our PD patients and CTRL individuals. IL-15 induces several chemokines in T lymphocytes, such as RANTES and MIPa (31). It also induces T-cell proliferation, activates cytolytic effector cells, and stimulates B cells to proliferate and secrete immunoglobulins. The characteristics of predominant expression of CD8(+)T cells, depletion of CD4(+)CD25+ cells, and a shift to a T(H)1-type immune response in the peripheral immune system in PD patients may reflect an immune reaction-associated inflammatory process in the brain (32). Several investigators have also described immunoglobulins that react with catecholaminergic tissue including autoantibodies against dopaminergic neurons in the cerebrospinal fluid (CSF) and serum from patients with PD (33, 34). The specific antigenic target of these immunoglobulins is mostly unknown. In a recent study, sera from parkinsonian patients exhibited a specific IgG response to dopamine-o-quinone-modified proteins, also known as quinoproteins (35). NK cells may induce cell death by attacking against antibody-coated dopaminergic neurons (36). Therefore, our results do not exclude the role of IL-15 in PD pathogenesis. Circulating cytokines have a short half-life, act mainly in an autocrine or paracrine fashion, may reach high concentrations at sites of release, but much lower concentrations after dilution in blood, or they may bind to molecules that do not permit their detection by immunological methods. All these may be the cause of the contradictory results of several studies that measured circulating cytokines in neurologic diseases. It is also possible that several inflammatory mediators may not be involved in a similar way in the pathogenesis of PD.

Gangemi et al. found a contemporaneous increase in circulating IL-15 and RANTES among patients with advanced stage of PD and severe functional impairment (37). In our study a strong correlation between serum RANTES levels and severity of the disease (UPDRS III score) was found. These results suggest that the disease stage may contribute to the expression of these molecules.

There are indications that dopamine may have some effects on the immune system. A recent review (38) indicates that this catecholamine, acting as a CNS transmitter, can influence the peripheral immune system perhaps through the synthesis or release of other neurotransmitters or regulators. Although dopamine has a much more limited role as a transmitter in peripheral tissues, the existence of dopamine and its receptors in peripheral tissues and cells suggests that it may have a more direct influence on the regulation of immune responses (39). Previous studies have indicated that mitogen-induced proliferation of lymphocytes, as well as cytokine synthesis such as interferon-γ may be regulated locally by dopamine (40–42). The specific effects and mechanisms are still unclear, however. l-Dopa-treated patients showed significantly higher IL-15 and RANTES circulating levels compared to healthy controls and higher, although not significantly, levels compared to untreated patients (37). Bessler et al. also showed that l-dopa caused an enhancement of IL-6 and TNF-α production by peripheral blood mononuclear cells from PD patients (43). The authors suggest that the immunologic alterations found in PD may be partially attributed to l-dopa administration. In our study l-dopa or dopamine agonist administration seemed to prevent the development of immunologic disturbances as serum RANTES levels from untreated patients were significantly elevated compared to CTRL subjects or treated patients. These data suggest a possible immuno-modulatory effect of l-dopa but it is not clear whether treatment acts as an immuno-stimulatory or as an immuno-suppressory factor.

With a larger sample size, circulating cytokines or chemokines in serum, and CSF or (and) cytokine levels in various regions of the CNS by immunohistochemical methods must be assessed for the peripheral or glial source of IL-15, RANTES, and other inflammatory mediators in patients with PD, and for clarifying the hypothesis of an association between the brain and the peripheral immune system.
References


40. Josefsson E, Bergquist J, Ekman R, Tarkowski A. Catecholamines are synthesized by mouse lymphocytes and regulate function of these cells by induction of apoptosis. Immunology 1996;88:140–6.

