Increased levels of IL-17A in patients with fibromyalgia

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ABSTRACT

Objectives. The aim of this study was to evaluate the plasma levels of IL-17A in fibromyalgia patients, and to look for any correlations between this data and the concentrations of some pro- and anti-inflammatory cytokines.

Methods. We performed a study including 58 fibromyalgia patients and 39 healthy women matched for age and body mass index. The plasma levels of IL-17A and other pro- and anti-inflammatory cytokines were measured by using the technique of cytometric bead array (CBA). The analysis of differences between groups was performed using Mann-Whitney test and the analysis of the correlations by Spearman’s correlation test.

Results. The analyses showed that fibromyalgia patients present increased levels of IL-17A. They also revealed that plasma concentrations of IL17A positively correlate with levels of IL-2, IL-4 and IL-10, TNF and IFNγ.

Conclusion. As far as we are aware, this is the first study to demonstrate increased levels of IL17A in fibromyalgia patients. The positive correlation between the levels of IL-17A and of other cytokines strengthens the hypothesis of the involvement of inflammatory mechanisms in the development of this syndrome.

Introduction

The aetiology and physiopathology of fibromyalgia (FM) remain unclear (1), although evidences point to the involvement of the immune system in its genesis and evolution (2-5). In this study, we analysed the participation of the immune system in FM by analysing the plasma levels of IL-17A, which is a potent pro-inflammatory cytokine (6), that has been implicated in the pathogenesis of some systemic inflammatory and autoimmune diseases (7-10). In addition, we have also looked for correlations between plasmatic levels of IL-17A and other pro- and anti-inflammatory cytokines.

Materials and methods

This study was conducted on 58 women in the FM group and 39 women in the control group. Although, the American College of Rheumatology (ACR) had published new criteria of diagnostic in 2010 (11), for this study FM was diagnosed according to the previous ACR criteria (12), since the stages of research planning and recruitment of patients began before 2010, and the new criteria of diagnostic are still controversial (13-15).

The study group included only patients with primary fibromyalgia. Patients diagnosed with FM with a history or presence of: chronic inflammatory conditions (e.g. spondyloarthritis and ankylosing spondylitis), autoimmune diseases (e.g. systemic lupus erythematosus and rheumatoid arthritis), psychiatric disorders (e.g. major depressive disorder, schizophrenic or paranoid disorder) and or presenting infectious were excluded from the study. Also excluded were patients who had used anti-inflammatory drugs in the past six months, pregnant patients or women who were breastfeeding. This study was approved by the ethics committees of UFMG (0224.0.203.000-10) and of UNIFOR-MG (158/2010). All patients and control subjects gave written informed consent before inclusion in this study. There were no statistically significant differences in age between the FM group (49.7±10.7 years) and the control group (50.3±7.7 years), and neither between the body mass index (BMI) in the FM group (26.3±4.5 kg/m²) versus the control group (26.7±2.4 kg/m²).

Cytokine levels were assessed using a commercial BD® Cytometric Bead Array (CBA) Tq/Tq2/Tq17 cytokine kit (BD Bioscience, San Diego, CA, USA) according to the manufacturer’s protocol, and the data were acquired.
using a FACSCalibur flow cytometer (BD Bioscience, San Jose, CA, USA). Standard curves were determined for each cytokine using a range of 0–5000 pg/mL. The quantity (pg/mL) of each respective cytokine was calculated using FlowJo 7.6.1 software (Tree Star™, Inc., Ashland, OR).

Ordinal data were analysed by Kolmogorov Smirnov, followed by a Mann-Whitney test for non-parametric data. The Spearman test was used for correlational analysis between variables. All data were analysed using GraphPad Prism 5.0 software (San Diego, CA, USA). The data were expressed as the mean ± SE. Significance was defined as $p \leq 0.05$ ($\alpha=0.05$).

**Results**

The levels of IL-17A were significantly higher in FM patients when compared to healthy controls (Fig. 1). Besides, the levels of IL-2, TNF, IFNγ and IL-4 were also significantly higher in FM patients, when compared to controls. No significant difference was observed in IL-10 and neither in IL-6 levels. Analyses of the Spearman correlation coefficient (Fig. 2) revealed significant positive correlations between IL-17A and IL-2 ($p=0.012$ and $r=0.353$), IL-17A and TNF ($p=0.003$ and $r=0.396$), IL-17A and IFNγ ($p=0.037$ and $r=0.295$), IL-17A and IL-4 ($p=0.037$ and $r=0.293$) and IL-17A and IL-10 ($p=0.002$ and $r=0.419$). There was no significant correlation between IL-17A and IL-6.

**Discussion**

This study is the first to demonstrate that FM patients present increased plasma levels of IL-17A when compared to healthy age-matched controls. IL-17A is mainly produced by $T_{h17}$ lymphocytes, but it can also be produced by natural killer cells, dendritic cells and neutrophils (16-18). The origin of IL-17A in FM patients was not addressed in this study, but it is possibly produced, at least in part, by activated $T$ lymphocytes, since we have previously demonstrated increased numbers of these cells in FM patients (5). We have also previously demonstrated increased levels of peripheral blood $CD5^+$ and $CD5^-$ B lymphocytes in FM patients (5), what could be induced by IL-17A, as have been suggested by studies on other inflammatory conditions (19, 20).

We also demonstrate here positive correlations between IL-17A concentration and other cytokines, such as IL-2, TNF and IFNγ, what strengthens the hypothesis of the involvement of inflammatory mechanisms in the development of this syndrome. IL-2 is able to stimulate the differentiation of $T_{reg}$
cells into T_{H}17 lymphocytes (21). TNF is also produced by T_{H}17 cells, and besides, it works in the stabilisation and maintenance of T_{H}17 cells (22). The association of TNF with IL-17A has been reported in several conditions characterized by chronic inflammation; these cytokines seem to act together in perpetuating the inflammatory process (10, 23). Regarding the IFNγ data, although some studies have demonstrated an inhibitory effect of IFNγ on IL-17A production (17, 24), there are others that show increased levels of both these cytokines, mainly in chronic inflammation and autoimmunity models (23, 25), likely because some cells, particularly B-cells, can co-produce them (22).

Positive correlations between IL-17A and IL-4 or IL-10, as were demonstrated here in FM patients, have also been described in some models of chronic inflammation and autoimmune diseases (26, 27). Although data from correlation analyses does not necessarily prove causation, it is tempting to speculate that our data from IL-4 and IL-10 levels could suggest an attempt of this cytokine to regulate the overproduction IL-17A and other inflammatory cytokines (28). Both IL-4 and IL-10 seem to be necessary for T_{reg} cell-mediated suppression of the T_{H}17 response (27).

It is known that changes in immune profile may influence the severity of main symptoms present in the FM (5, 29), and that an imbalance between pro- and anti-inflammatory cytokine levels could explain, at least in part, the induction and maintenance of symptoms.

Fig. 2. Correlation between plasma levels of IL-17A vs. plasma levels of: IL-2 (A), IL-4 (B), IL-6 (C), IL-10 (D), TNF (E) and IFNγ (F) in FM patients. The significant correlations (p) and Spearman’s correlation coefficient (r) are shown on their respective charts.
in FM patients (30, 31). Moreover, it has been shown, in other clinical conditions, that IL-17A positively correlates with indices of pain (32), depression (33) and anxiety (34), which are symptoms frequently reported by patients with FM.

Finally, we would like to emphasise the relevance of this study as being the first to demonstrate that fibromyalgia is associated with increased levels of IL-17A. The data from correlation analyses between the levels of IL17A and levels of other cytokines strengthens the hypothesis that point to the involvement of inflammatory mechanisms in the development of this syndrome. Within this perspective, future studies could be performed to clarify the effects of specific anti-inflammatory therapies of IL-17A in the quality of life and in symptoms presented by FM patients.

References

References

Inflammatory cytokines in fibromyalgia patients / A.P. Pernambuco et al.